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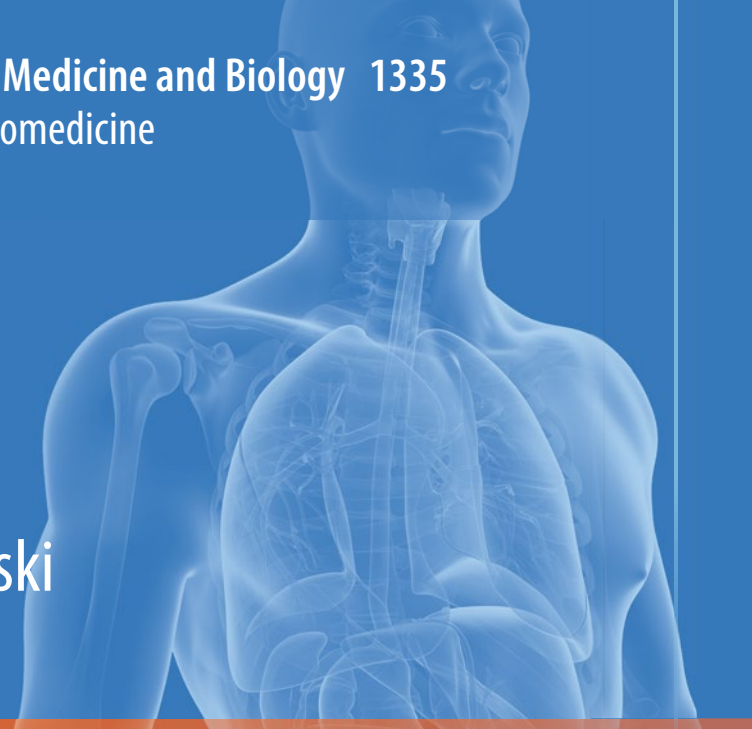

Mieczyslaw Pokorski
Editor

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Contents

Artificial Intelligence in the Healthcare System: An Overview 1
Jacek Lorkowski, Oliwia Grzegorowska, and Mieczysław Pokorski

Complex Interaction Among Immune, Inflammatory, and Carcinogenic Mechanisms in the Head and Neck Squamous Cell Carcinoma 11
Ana Caruntu, Cristian Scheau, Mircea Tampa, Simona Roxana Georgescu, Constantin Caruntu, and Cristiana Tanase

Outcome of Repeat Pulmonary Metastasectomy 37
Marcus Krüger, Katharina Franzke, Taufiek Konrad Rajab, Fabian Nadler, Moritz Möbius-Winkler, Norman Zinne, Daniel Schulz, Miriam Möller, Wolfgang Schütte, Michael Ermitsch, Bassam Redwan, Olaf Schega, and Christian Biancosino

Quality of Life of Patients After Laparoscopic Pyeloplasty Due to Ureteropelvic Junction Obstruction: A Long-Term Observation 45
Wojciech Panek, Dawid Janczak, Marta Panek, Urszula Szydełko, Rafał Chrzan, Mariusz Chabowski, and Tomasz Szydełko

Causes and Effects of Introducing Surgery Safety Checklist: A Review 53
Jacek Lorkowski, Izabella Maciejowska-Wilcock, and Mieczysław Pokorski

Benefit of Biological Drugs for Quality of Life in Patients with Ankylosing Spondylitis: A Systematic Review and Meta-Analysis of Clinical Trials 63
Wojciech Tański, Natalia Świątoniowska-Lonc, Krzysztof Dudek, and Beata Jankowska-Polańska

Changes of Motile Ciliary Phenotype in Patients with Primary Ciliopathies 79
Miroslava Brndiarova, Julia Kvassayova, Jarmila Vojtkova, Matus Igaz, Tomas Buday, and Jana Plevkova

Effects of Inhalation of STIM-Orai Antagonist SKF 96365 on Ovalbumin-Induced Airway Remodeling in Guinea Pigs 87
Martina Šutovská, Michaela Kocmálová, Ivana Kazimierová, Christina Imnoy Nøss Forsberg, Marta Jošková, Marian Adamkov, and Soňa Fraňová

***In Silico* Analysis of Bone Tension During Fixation of the Medial Malleolus Fracture After Ankle Joint Endoprosthesis** 103
Jacek Lorkowski, Renata Wilk, and Mieczysław Pokorski

Effects of Physical Activity at High Altitude on Hormonal Profiles in Foreign Trekkers and Indigenous Nepalese Porters 111
Alessandro Tafuri, Danilo Bondi, Alessandro Princiotta, Tiziana Pietrangelo, Pabitra Yadav, Vincenzo Maria Altieri, Maria Angela Cerruto, Fiore Pelliccione, Alessandro Antonelli, and Vittore Verratti

Influence of Parathyroidectomy on Sleep Quality in Primary Hyperparathyroidism 121
Renan Viola Rasche, Frauke Schuster, Natalie Meurer, Theodora Margariti, Norbert Weyerbrock, Kurt Rasche, and Cornelia Dotzenrath

Mini Craniotomy in the Management of Supratentorial Spontaneous Intracranial Hemorrhage: A Single-Center Outcome of the Minimally Invasive Treatment 129
Binod Bhattarai, Aliza Bajracharya, Suja Gurung, Sweta Giri, Sashi Bhusan Sah, and Sunil Munakomi

Correction to: Outcome of Repeat Pulmonary Metastasectomy C1

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Artificial Intelligence in the Healthcare System: An Overview

Jacek Lorkowski, Oliwia Grzegorowska, and Mieczysław Pokorski

Abstract

This chapter aims to present insights into the influence of artificial intelligence (AI) on medicine, public health, and the economy. PubMed and Google Scholar databases were used for the identification and collection of articles with search commands of "artificial intelligence" AND "public health" and "artificial intelligence" AND "medicine". A total of 273 articles specifically handling the issue of artificial intelligence, dating ten years back, in three major medical journals: *Science*, *The Lancet*, and *The New England Journal of Medicine*, were analyzed. Computational power gets stronger by the day, giving us new solutions and possibilities. Current medicine problems like personalized medicine, storage of data, and documentation overload will likely be replaced by AI shortly. The application of AI may also bring substantial benefits to other areas of medicine like the diagnostic and therapeutic processes. The development and spread of AI are inescapable as it lowers healthcare and administrative costs, improves medical efficiency, and predicts and prevents major disease complications. The use of AI in medicine seems destined to carry the day.

Keywords

Artificial intelligence · Healthcare system · Medical documentation · Medicine · Public health

1 Introduction

Astronomy is probably the first field in which openness in sharing the knowledge brought about the milestone progress for man as the notes of Chinese astronomers dated to 1054 AD enabled Carlo Otto Lampland to discover a remnant of the supernova Crab Nebula in 1921 (Fore 2019). The faith in the progress achieved through cooperation and parallel learning helps achieve success, even if it is a collaboration between distant generations. Likewise, in high-energy physics, access to open data is essential for further discoveries and scientific progress. Large international scientific organizations, for instance, the

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European Council for Nuclear Research (CERN), founded in Europe in 1952, have become world-class research bodies by sharing knowledge with the global community. This is a progressive way of science practice based on cooperation rather than pure competition posed to bring about great discoveries.

A steady increase in the amount of data to be converted into knowledge enforces adequate software solutions, workflow, and explanations (Chen et al. 2019). To process petabytes of data, it is of the highest need to implement solutions of artificial intelligence (AI) because the work cannot be performed by a man (Lorkowski and Malinowska 2020). The introduction of such solutions is unavoidable, especially during the SARS-CoV-2 pandemic, when the time seems increasingly essential. The pandemic has hastened up this process. The AI and deep neural networks (DNN), designed for the analysis and processing of tremendous volumes of data, appear of critical help in drug or vaccine discoveries, observation of clinical effects, and further outcomes (Grzegorowska and Lorkowski 2020).

The 1970s can be considered as the onset of AI and knowledge sharing ideas in the modern world. The oil crisis of the time forced Jack Niles to propose the terms "teleworking" and "telecommuting" to reduce fuel consumption in the United States. Based on this innovative concept, Alvin Toeffler raised the idea of an electronic village as the main place of production in the future. The idea was broadly discussed in Europe, especially in Scandinavian countries, but the concept of telework was nearly forgotten. Then, it came back to life in the 1980s and is steadily on the rise since (Dangelmeier et al. 1999). The current SARS-CoV-2 pandemic situation has made this concept come true as people have started working from home due mostly to social distancing.

Biomedical science focuses on four interrelated topics: personalized medicine, data-intensive technologies, big data and information technologies, and AI (Schork 2019). This chapter aims to present a short insight into connections between artificial intelligence, medicine, public health, and the economy. To this end, we screened PubMed and Google Scholar databases using the commands of "artificial intelligence" AND "public health" and "artificial intelligence" AND "medicine". As of December 1, 2020, the search returned 18,000 and 11,000 entries concerning either combination terms, respectively, dating ten years back. From this huge volume of articles, we chose for further analyses those which specifically handled the issue of AI and were published in the following three renowned medical journals: 185 articles in *Science*, 61 in *The Lancet*, and 27 in *The New England Journal of Medicine*.

2 Public Health and Smart Cities

Charles Edward Amory Winslow of Yale University in New Haven, Connecticut, proposed the following definition of public health in 1920: "Public Health is the science and the art of preventing disease, prolonging life, and promoting physical health and efficiency through organized community efforts for the sanitation of the environment, the control of community infections, the education of the individual in principles of personal hygiene, the organization of medical and nursing service for the early diagnosis and preventive treatment of disease, and the development of the social machinery which will ensure to every individual in the community a standard of living adequate for the maintenance of health; organizing these benefits in such fashion as to enable every citizen to realize his birth right of health and longevity" (Winslow 1920). Public health uses specific methods and concepts to measure and describe health conditions. A widely used concept is the quality of life (QoL) is defined by the WHO as "individuals' perception of their position in the life in the context of the culture in which they live and in relation to their goals, expectations, standards, and concerns". The QoL is commonly evaluated in patients with cancer, mental illness, heart disease, gastrointestinal disease, chronic obstructive pulmonary disease, asthma, and in the elderly (Haraldstad et al. 2019). Public health sciences

also explore how urbanization affects human life and health and to what extent citizens can influence their living conditions. It is known that city-dwelling relates to a better QoL, physical health, access to the healthcare system, as well as enabling the elderly to have better social and physical activities (Zagozdzon et al. 2011; Eggebeen and Lichter 1993). Such factors appear to entice people to dwell in big cities or their neighborhoods, which spurred the development of the 2003 New Charter of Athens, an innovative project connected to urbanization (Stouten 2003). From the healthcare standpoint, the purpose of such initiative has been to form a national health-promoting living space for residents by the reorganization of urban planning, inclusive of an entire range of public health services based on the use of information and communication technologies (ICT) (Lorkowski and Malinowska 2020). The ICT is involved in the prevention of life-threatening situations like cardiorespiratory arrest, cardiac and brain infarcts, accidental falls suffered by the frail elderly, and others requiring sophisticated and prompt help. These situations shape the idea of a smart city proposed by the International Communication Union and defined as "an innovative city that uses the ICT and other means to improve QoL, the efficiency of urban operations, and services, and competitiveness while ensuring that it meets the needs of present and future generations with respect to economic, social, and environmental aspects" (Toh et al. 2020). It follows that the use of AI is naturally integrated into the concept of a smart city. The United Kingdom, China, and India are the countries that have allocated substantial fiscal resources to design and implement smart cities (Toh et al. 2020).

The 2017 Global Smart City Performance Index, evaluating the four most important vectors in the city function such as mobility, healthcare, safety, and productivity, was topped by Singapore followed by London and New York (Smart Cities Association 2017). These cities ranked best on the number of hospital beds per capita, road security, air pollution, bicycle communication, public transport, telemedicine, digital health portals, virtual medical advisor assistance, elderly care, and campaigns promoting healthy ways of life. There are reports that each citizen "gets back" 15 days' worth of time every year owing to the ICT. This time can be spent on physical activity or sleep or adoption of healthy eating habits, which all positively affect cardiocerebrovascular health (Riggs et al. 2018; Seixas et al. 2018). Physical activity reduces weight and glucose level and improves the quality of sleep, making also chronic obstructive pulmonary disease (COPD) patients benefit from it (Lewthwaite et al. 2017). The Global Liveability Index (2021) created by *The Economist* ranks 140 major cities in the categories of stability, culture and environment, education, infrastructure, and healthcare. Healthcare is evaluated in the smart city context by assessing both public and private systems. In 2018, the maximum score in the index was achieved by the cities of Vienna, Melbourne, Osaka, Calgary, Sydney, Vancouver, Toronto, Tokyo, and Adelaide.

On the other hand, the contemporary SARS-CoV-2 pandemic points out some limitations inherent to the smart city concept. One of them is the problem of communication systems as they are fragmented and often understood only by service providers. An effort should be made to standardize and find the best solution to cooperate in the case of disasters and comprehensively build cities regarding public health, particularly when the situation enforces social distancing or other extraordinary measures (Allam and Jones 2020; Capolongo et al. 2020).

3 Artificial Intelligence (AI)

AI is used in various fields, from public health to biology, and can be reduced to the task of predicting an outcome from diverse features or finding repeating patterns in large datasets (Deo 2015). Today, AI is mostly applicable in machine learning (ML), also in medicine. There are three kinds of ML—supervised, unsupervised, and reinforcement learning. When the first one focuses on the classification of the data, the second is trying to find unique patterns and assess their viability. The patterns are then evaluated in

supervised learning tasks. Reinforcement learning is reward-based learning used mostly in robotic applications (Aktolun 2019). The most widely used method is supervised learning that uses the following algorithms: linear and logistic regressions, artificial neuron networks (ANN), support vector machines (SVM), and tree-based methods; all can be combined. A special subset of ML is the deep learning that builds the ANN, the process inspired by neural interconnections in the human brain and consisting of multiple layers made of nodes. The nodes are interconnected with nodes in the back and forward layers as it takes place in the brain. In practice, these methods help analyze huge amounts of data in a chosen context. The SVM builds a model that assigns new items to a category in the linear classification. Applying a kernel trick allows making nonlinear classifications by mapping their inputs into high-dimensional feature spaces. Finally, a decision tree is the most common method that reconstructs the tree structure and assigns labels by creating appropriate "splits" (Al'Aref et al. 2019).

AI is a strong technological sequence providing sources of machine deduction, reasoning, learning, and interaction. It has entered our everyday life by solving business problems sensibly and acceptably, including building advanced algorithms, analyzing data, enhancing the computational power and storage, and lowering costs (Ergen 2019). Despite these facts, AI has been portrayed by Stephen Hawking as a possible threat to the world economy as it may one day replace humanity. Also, people should focus on creating a beneficial AI rather than any AI, which is of essential importance for AI not to become a digitalized utopia (Hawking 2015).

4 Blockchain Technologies

The WHO has proposed a classification of digital healthcare services into interventions for clients, healthcare providers, health system or resource managers, and data services. We are currently a part of digital transformation where digital medical networks can be created using two different concepts. The first concept assumes the use of a centralized network, a technology tested during the First Gulf War and then used by corporations such as IBM and Walmart, which substantially increased profits and helped improve the quality of customer services. Referring to the medical field, a basic issue is to develop a centralized network that would be a fast and effective information exchange platform that ensures the security of data (Lorkowski and Malinowska 2020). The second concept, which gains popularity and progresses promptly, relates to the development of cryptocurrencies. It is the blockchain technology that enables transactions between two or more parties without a centralized authority and secures them with cryptographic principles making them inexpensive and fast. This technology can be defined as a chain of blocks with time stamps connected through cryptographic hashes. The chain can grow all the time as new blocks are added, with each new block containing a link to the content of a preceding block's link. The blockchain, called the distributed general ledger, is the distributed database, also known as the registry or common register. Each piece of information is stored as an independent copy of the registry on computers and servers around the world. It means that every user has its copy of all collected data (Kuo et al. 2017).

Key benefits of the blockchain, when compared to traditional databases for biomedical and healthcare applications, include decentralization and cryptography providing security and privacy in addition to immutability (data once saved cannot be changed, corrupted, or retrieved), assignment of health data to the patient, and data robustness, transparency, trust, and verifiability. This technology is used in electronic health records (EHR), remote patient monitoring, and pharmaceutical industries, where distributed data ledgers may improve the management of medical records and accelerate biomedical research and education. These fields also are vulnerable to potential threats of forgery.

Currently, a more patient-centered connection of different, previously independent noncommunication systems is needed, followed by transformation into reliable electronic databases. The

development of blockchain networks in medicine is determined by connections between particular parts of the system. A major drawback is that these networks are created by different suppliers and based on different solutions, which creates compatibility and standardization problems. Another challenge is to adequately protect medical data against potential security breaches. Moreover, the European Union General Data Protection Regulation assumes that the user always has a right to request complete erasure of their data. The blockchain technology, on the other hand, makes it impossible due to immutability. Finally, very large volumes of data may degrade the processing performance by lowering the system's speed (Agbo et al. 2019).

5 Sensitive Data

A basic issue concerning a large collection of data and AI in medicine and public health is database safety. As data in medicine contain racial, genetic, biometric, and even religious belief information and evidence of identity, they are regarded as sensitive. That does not exclude the anonymous use of data, like in meta-analysis, which is a very worthwhile cause. Fortunately, the unauthorized use of sensitive data is unlikely when proper security measures are adopted. It happens some online applications have racial and socioeconomic denigrating content, which meets with public outcry. Such is usually introduced onto platforms through the commonly anonymized unfiltered data streaming from the Internet. Luckily, there have been no similar incidents in the healthcare system. However, further watchfulness and testing the security measures are required before full implementation of AI in healthcare (Al'Aref et al. 2019). Researchers argue that anonymization of medical data, particularly identity, is so well advanced that the security breach is less likely than gaining obtaining information from within the system due to the vulnerability of hospital records to external hacking. It means that the weakest link in the system may be a man. (Lorkowski and Malinowska 2020; Mearian 2018). The first AI-based and US Federal Drug Administration (FDA)-approved application for facilitating clinical, particularly cardiovascular, diagnoses, called the Arterys medical imaging platform, provides a unique solution for the Protected Health Information (PHI) service. The application splits out data that may be responsible for the evidence of identity and then stores them in the Arterys cloud and the hospital PHI secure server. These data can be later rebuilt when an accredited user logs into the system (Marr 2017).

Medical data are a potential economic resource for individuals, companies, and countries. The issue of sharing medical data remains unresolved as they should be processed in compliance with the highest ethical standards. Plausibly, a worldwide collection of medical data might be the most valuable and largest bunch of data humanity possesses. It might also become a potential global resource of strategic importance for humanity's development and prosperity. It all demands a special infrastructure, legal conditions, and making ways of collecting, processing, and storing of data, remembering to deal with the population data, not concerning individuals.

6 Medical Documentation

The volume of medical documentation increases every year, taking doctors' time away from the direct care of patients. This takes a toll on both physicians and patients and also increases the costs of medical services (Raulinajtys-Grzybek and Lorkowski 2020). A burnout syndrome, on the increase in physicians, appears connected with the time spent on filling and creating medical documentation. It has been estimated that each hour spent with a patient elates to two hours spent on EHR, mostly on documentation. In the United States, a "wasted" has been priced at 90–140 billion dollars in lost physician productivity every year (Lin 2020). It is known that there are two possibilities for creating and storing medical documentation: traditional on paper and digital. It is most probable that a mix of the two will persist for a long time to come. An unfortunate underestimation of the advantages of digitalized documentation is felt, which is an

attempt to deny the laws of high numbers and statistics (Bloom and Cadarette 2019).

It is estimated that about 91 billion dollars (14% of public health spending) were wasted due to invalid administration in 2018. That pointedly shows that the current model of appointing principals and tasks for health bureaucracies simply does not work. Applying AI to provide an appropriate execution of operations and optimization of procedures should contribute to reductions in costs and lost opportunities. The excessive paperwork is a consequence of unreasonable and often reprised document circulation. Jonathan Bush has described this phenomenon as "sewage" of modern medicine, and applying AI for the analysis of bureaucratic procedures will reduce, improve, and redirect to the useful operational tracks. A reorganization of funds allocation, reduction of costs of lost opportunities, and the optimal cash flow are the most expected results of these actions. For many observers, the hitherto system of medical documentation in healthcare is seen as a huge, monolithic, difficult to use, and reform-inert structure. Paradoxically, the world's most popular systems of archiving data are often built based on old technologies that have been primarily dedicated to building first databases. That might be a reason they seem more intuitive for an average user (Bush and Baker 2014).

In an article published by *The New Yorker*, the implementation and evaluation of new software are time-consuming. Additionally, it needs constant improvements. A software system described was created not for the medical staff but for patients who would like to check their laboratory results, remember the drug list, or track their conditions. Any step forward toward more convenient patient-oriented management is beneficial from the medical and economic standpoints as the chances of health improvement and prompt return to work increase (Gawande 2018).

Creating the EHR helps streamline the patient visit. However, EHR affects the patient-physician communication in complex ways, enforcing changes in the consultation attitude that would satisfy both sides (Crampton et al. 2016). Medical Scribe services gain popularity, offering a smooth transition from speech into a written text, performing documentation in the EHR, and partnering with the physician to deliver the most efficient patient care (Ash et al. 2020). There also exist government-sponsored EHRs introduced to collect medical data from a wide range of people. Their main purpose is to monitor long-term clinical and public health patterns that might be therapeutically and epidemiologically useful for the future. The Biobank in the United Kingdom and the NIH *All of Us* Research Program in the United States of America change the practice of personalized medicine by collecting and evaluating data from millions of people to accelerate research, diagnosis, and treatment services (Bycroft et al. 2018; Sankar and Parker 2017).

Medical data are specific and heterogeneous and come in a variety of forms that are essential for proper identification of patient health status. Machine learning, which is capable of extracting information from data, is of substantial help in medical care. Large amounts of collected data require special processing to be introduced into AI algorithms that must be able to validate the information before its clinical adoption. That is why learning from and making predictions on data requires to split them into training, validation, and test sets. Test data should be prepared in a specific objective way, contain data from various hospitals and institutions, and used as often as possible (Park et al. 2019).

7 Multiomics, Precision, and Personalized Medicine

The biological identity is made of features characterized by individual epigenomics, genomics, metabolomics, microbiomics, pharmacogenomics, proteomics, and other "omic data", which all create a "permutome". The field has been called "multiomics" (Livingstone et al. 2015). The "omics" can be used to create personalized diagnostic and treatment procedures and increase their efficacy and safety. Precision medicine uses multiomic data to improve human health, better understand human biology, and

create a perfect healthcare system that would be predictive, preventive, personalized, and participatory ("P4") (Ziegelstein 2017a; Guzzi et al. 2016). Most implementations of genomic profiling can be classified into one of two models based on the outcome: (1) research to discover generalized knowledge and (2) search to recover individualized knowledge (Trent 2019). A perfect example of therapy based on recovering individualized knowledge is chimeric antigen receptor T-cell therapy (CAR-T) using T cells grown in the laboratory to treat various types of cancer (Graham et al. 2018).

In the past, medicine has been practiced without evidence-based knowledge but medical care was adjusted to the individual. Current medicine has been dominated by technology, electronic documentation, and a race of time that often pulls the doctor away from the full care for patients. A question arises of effective doctor–patient communication. Ziegelstein (2017b) emphasizes that therapy, based on created guidelines, is tailored to groups rather than to individual patients, which makes the patient invisible in a group with similar diseases and characteristics. A new term has been adopted in clinical practice—"personomics"—to describe this unique phenomenon. Personalized medicine uses the knowledge about the patient's values, goals, preferences, and financial resources, all of which are included in "personomics". As Ziegelstein (2017b) states, "The evolution from precision medicine to personalized medicine is the evolution from healthcare to health caring". On the other hand, Jameson and Longo (2015) state that since medicine has been individualized and personalized, the term precision medicine should also consider the patient's psychosocial status. AI has strongly influenced personalized medicine. Consequently, the best possible solution for connecting personalized medicine with healthcare is to create a single process in which diagnosis, treatment, and follow-up of the patient are supplementary to each other and enable a fluent transition from one activity to another (Schork 2019). As the primary goal of precision medicine is developing models for humanity to predict the health status and prevent disease and disability, its application without using AI to process large data collection is next to impossible.

8 Artificial Intelligence (AI) in Medicine

The AI is tested in increasingly large medical fields. The first recognition came with the discovery that the computer-aided reading of mammograms is equal to the reading by a physician (Gilbert et al. 2008). Since that time, a lot of articles have described the application of AI in radiology and other fields of medical imaging, for example, pulmonology, invasive and noninvasive cardiology, psychiatry, or orthopedics (Kalmet et al. 2020; Refaee et al. 2020; Al'Aref et al. 2019; Davatzikos 2019; Betancur et al. 2018). Precision medicine offers a broad application of deep learning methods in defining features responsible for or predisposing to various diseases such as Alzheimer's, schizophrenia, dilated cardiomyopathy, or heart failure (Lin and Tsai 2019; Shah 2017). Genomic studies also amply use deep learning methods to name variants of protein sequences, predict mutation effects, or identify binding motifs (Bao et al. 2020). Of notable interest is a "deep patient" presented by Miotto et al. (2016), who have used the EHR to further clinical predictive modeling and observed substantial improvements in the diagnosis of testis and prostate cancers, sickle-cell anemia, attention deficit, and disruptive behavior disorders.

The proposed solutions influence the perception of modern medicine. The AI, whose increasing use is inescapable, substantially reduces healthcare costs. It helps doctors make their work and develop skills instead of spending time filling out medical records. The acceptance of AI in everyday life cannot adversely affect procedural outcomes and clinical efficacy. Conversely, it provides computational suggestions and models to avoid medical complications. The AI also makes it possible for doctors to extend the years of their active work time, with the accompanying advantages for the gross domestic product.

9 Conclusions

AI has undoubtedly improved medical care and management. Computer-based algorithms are time- and cost-effective measures that show the likely direction of future transformative developments (Anderson 2019). Innovative strategies in healthcare should center on the patient's well-being. Electronic health records help personalize medical care using technology as a consolidative tool. The AI is fundamental for the medical practice of excellence (Souza Filho et al. 2019). On the other side, there may be limitations for AI use in medicine. Some medical professionals harbor a bit of prejudice toward machine learning methods. Where the precision counts most, like in a healthcare system, there can be some selection pitfalls. The sampling and observer selection bias, indifferent in most commercial settings, can adversely affect the process of medical modeling. Selection bias can occur when an algorithm concludes deeply flawed data (Al'Aref et al. 2019). The occurrence of some diseases can be underpredicted due to inadequate screening, epidemiologic, or other poorly controlled factors. Such issues are difficult to identify and remedy in clinical trial datasets based on machine learning methods. Nonetheless, progress in the development of AI holds humanity's future in many a field, including resource efficiency, autonomous machines, healthcare, and the like. The AI is not likely to replace a man in many professions, but the expectation is that it will create more jobs than destroy, increasing productivity at the same time. As Ergen (2019) states, "The industrial era let machines do the physical work, the information era enabled machines to do the computation and storage, now the AI era will let machines make the decisions".

Conflicts of Interest The authors declare no conflicts of interest concerning this chapter.

Ethical Approval This chapter does not contain any studies with human participants or animals performed by any of the authors.

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Complex Interaction Among Immune, Inflammatory, and Carcinogenic Mechanisms in the Head and Neck Squamous Cell Carcinoma

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Abstract

Inflammation is deeply involved in the development of most types of cancer. Many studies focus on the interaction between immune-inflammatory mechanisms and tumorigenesis in the head and neck squamous cell carcinoma (HNSCC). In this chapter, we emphasize the complexity of processes underlying this interaction and discuss the mechanisms of carcinogenesis in HNSCC with a special focus on metabolic changes, inflammation, and the immune landscape. Unveiling complex connections between immuno-inflammatory processes and tumor initiation, promotion, and progression will open new directions in the reliable identification of predictive factors and therapeutic targets in HNSCC.

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Keywords

Cancer · Head and neck squamous cell carcinoma · Immunity · Inflammation · Therapy · Tumorigenesis

1 Introduction

Inflammation is deeply involved in the development and progression of most cancers, even if, at first glance, the mechanisms of inflammation are not prominent actors (Taniguchi and Karin 2018). Inflammation has been recognized as a promoter at all stages of tumorigenesis (Greten and Grivennikov 2019). Hence, it is not surprising that researchers have focused on studying the relations between immune-inflammatory mechanisms and tumorigenesis in different types of cancer, including head and neck squamous altering the cell carcinoma (HNSCC) (Georgescu et al. 2020; Scheau et al. 2020; Neagu et al. 2019; Tampa et al. 2018a; Tampa et al. 2018b; Georgescu et al. 2017; Neagu et al. 2016).

HNSCC is the world's sixth most frequent cancer, with an increasing incidence during the last decades, as life expectancy and global population increase (Economopoulou and Psyrri 2017). Despite great progress in diagnosing and treating cancers, death rates associated with HNSCC did not decrease significantly, due most likely to a high rate of the first diagnosis in advanced stages of the disease (Jou and Hess 2017). When early diagnosed and treated, survival rates in patients with HNSCC exceed 80–90% (NIH 2017). When distant metastases occur, although rare compared to other cancers, a high death toll of 74% at 24 months is present (Wiegand et al. 2015). This condition is commonly diagnosed in adult and elderly population, but recent data show a decreasing trend of the onset age. Men are three times more likely to develop HNSCC, but lately, an increasing incidence has also been reported in women (Chi et al. 2015).

2 Etiopathogenic Features of HNSCC in Different Locations

HNSCC includes a series of malignancies with different anatomical locations, for example, lip, oral, sinus cavities, pharynx and larynx, which are grouped according to etiopathogenic similarities, as well as clinical, therapeutic, and evolution criteria (Faraji et al. 2017).

Various endogenous and environmental factors participate in the development and progression of regional HNSCC (Solomon et al. 2018; Wu et al. 2018; Lupu et al. 2017; Voiculescu et al. 2016). For example, ultraviolet (UV) radiation is the main risk factor for lip and skin SCC as damaged. Keratinocytes accumulate mutations (Lupu et al. 2020; Chan et al. 2019; Lupu et al. 2018a, b). The UV exposure is also associated with the release of pro-inflammatory cytokines, such as TNF-α, stimulating the differentiation of monocytes into macrophages and maintaining local inflammation, which may induce angiogenesis and tumor progression (Bottomley et al. 2019). Moreover, inhibition of antitumor response in CD4+ and CD8+ lymphocytes against keratinocytes with alterations induced by UV radiation has been reported (Suwanpradid et al. 2017). Exposure to UV radiation has also been investigated in the context of decreasing density of the antigen-presenting. Langerhans and dendritic epidermis-resident cells. The apoptosis of these cells may exert Th2 and Treg-related immunosuppressive effects altering the antitumor immune response (Otsuka et al. 2018; Pettersen et al. 2011).

In HNSCC, smoking and chronic alcohol consumption are involved in more than 75% of cases (Hashibe et al. 2007). When these factors are combined, their damaging effects are enhanced and the risk of developing HNSCC can increase by 35-fold (Economopoulou and Psyrri 2017). Tobacco use induces epigenetic alterations in oral mucosa, with modified expression of various

genes such as p53, GLUT-1, p16, and P13K in oral epithelial cells. It can impair various immune mechanisms involved in antitumor response and can activate oxidative stress reactions, leading to increased expression of pro-inflammatory genes and status of chronic inflammation (Jiang et al. 2019; Khowal and Wajid 2019; Seifi et al. 2014). Alcohol increases the permeability of oral mucosa for carcinogens. It also generates toxic compounds that can induce DNA damage and stimulate cell proliferation. Chronic alcohol intake is also associated with free radicals production that may induce immunosuppression (Feller et al. 2013).

Human papillomavirus (HPV) infection is mainly associated with oropharyngeal cancers, exhibiting a different behavior compared to tobacco- and alcohol-induced cancers (Tampa et al. 2020; Boda et al. 2018; Georgescu et al. 2018; Kobayashi et al. 2018; Boda et al. 2016). Almost half of these carcinomas are correlated with an active HPV status (Quabius et al. 2015). The most prevalent viral types are HPV16, HPV18, and HPV33 (Faraji et al. 2017; Ndiaye et al. 2014). Studies on a large number of patients have suggested that HPV+ tumors should be considered as distinct pathological entities within the wide group of HNSCC due to specific molecular features that correlate with improved response to therapy and prognosis (Dayyani et al. 2010). The 2017 classification of the tumor (T), nodes (N), and metastases (M) (TNM) has been updated according to these findings (Amin et al. 2017). Moreover, individualized therapies are proposed based on the HPV status (Cao et al. 2017; Husain and Neyaz 2017; Keck et al. 2015). There is a complex connection between HPV infection and chronic inflammation as the inflammatory cytokines can facilitate viral penetration and survival in the oral mucosa. They can also influence the proliferation of keratinocytes and viral activation (Tezal 2012; Tezal et al. 2012). Other studies have shown that HPV infection is associated with an increased T-cell infiltration and immune cell activation, considering the HPV status as a predictive factor for programmed cell death protein 1 (PD-1) inhibitors' therapeutic efficacy in HNSCC (Wang et al. 2019; Gameiro et al. 2018).

Occupational exposure to carcinogens like wood, textiles, or leather compounds is incriminated in the development of sino-nasal SCC. These tumors have a poor prognosis, especially when correlated with smoking, considering that most patients show advanced disease staging at the time of diagnosis (Elgart and Faden 2020).

A glance at the main risk factors of HNSCC and their mechanisms of action brings to attention the interconnections between inflammation, immune factors, and carcinogenic processes (see Fig. 1). To unravel the complexity of these processes, in the following sections we delineate the mechanisms of carcinogenesis in HNSCC with a special focus on metabolic changes, inflammation, and the immune landscape. We also highlight the importance of this topic for the discovery of new biomarkers and emerging therapies for HNSCC.

3 Mechanisms of Carcinogenesis in HNSCC

3.1 Genetic Traits in HNSCC

The mechanism of cancer initiation has been intensely studied over the years. The first step in the understanding of carcinogenesis was Rudolf Virchow's description of cancer cells (see McManus 1958). Shortly afterward, Theodor Boveri described the uncontrolled division of cells with a modified nuclear content in cancers, and these changes were linked to external factors (see Opitz 2016). Decades later, the first oncogene v-Src and the first tumor suppressor gene (TSG) were discovered, confirming the genetic basis of carcinogenesis (Alfred and Knudson 1971; Duesberg and Vogt 1970). Experimental murine models have been created through genetic engineering over the last years, with the intent of studying various types of cancer (Kersten et al. 2017). DNA sequencing techniques have revolutionized medical research allowing the characterization of the entire genome, including the mutations that trigger tumors (International Human Genome Sequencing Consortium 2004). Focal mutations, deletions, duplications, or

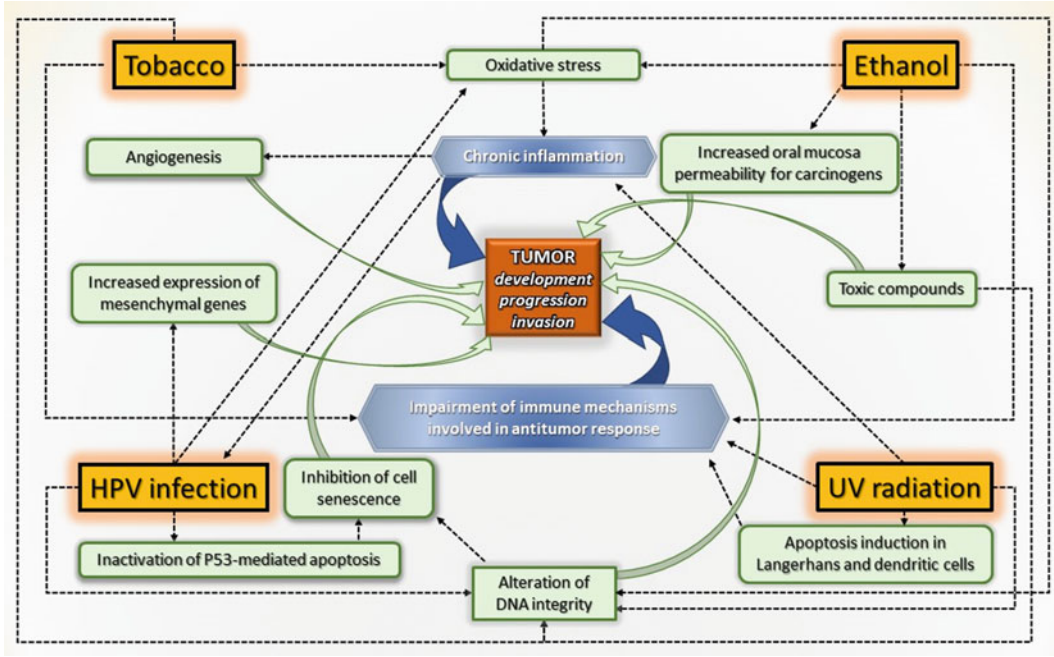

Fig. 1 Interconnections between inflammation, immune factors, and carcinogenic processes associated with the main risk factors of head and neck squamous cell carcinoma (HNSCC)

insertions of nucleotides contribute to the tumor mutational burden (TMB). A genetic database analysis has identified patterns of mutated genes, attributing genetic signatures to each type of neoplasia (Fancello et al. 2019; Chalmers et al. 2017).

HNSCC arises frequently from premalignant lesions, with a gradual accumulation of mutations associated with long-time exposure to external carcinogens in addition to increased age-related DNA instability (Monisha et al. 2017). Mutations of the tumor suppressor TP53 gene have been identified in most cancers (Perri et al. 2016) and in more than 70% of HNSCC (Pérez Sayáns et al. 2019). Labeled "guardian of the genome", the TP53 acts through multiple intracellular signaling pathways controlling processes like DNA repair, cell cycle arrest, and apoptosis. A transition to altered phenotype induces resistance to apoptosis, genome instability, and decreased immunogenicity (Perdrix et al. 2017). In nicotine-induced HNSCC, TP53 mutations with loss of function are almost constantly present (Cancer Genome Atlas Network 2015), even from very early stages, affecting guanosine nucleotides (Denaro et al. 2011). Deletions in the tumor-suppressing CDKN2A gene are in the top five most frequent mutations in the smoking-induced HNSCC. When associated, mutations in CDKN2A and TP53 genes negatively impact survival (Pérez Sayáns et al. 2019).

In contrast, HPV-positive tumors exhibit a low mutational burden involving these two suppressor genes. The overexpression of specific viral HPV E6/7genes, amplification of PIK3CA and E2F1 genes, and a loss of TRAF3 are the most representative genetic changes (Cancer Genome Atlas Network 2015). HPV infection, acting through viral oncoproteins E6 and E7, inactivates tumor supressor genes pRb and p53 in the host cell, leading to a loss of cell-cycle control, chromosomal instability, impaired DNA repair, and alterations in cell senescence and apoptosis (Castellanos and Pan 2016; Kim 2016; Doorbar et al. 2015). The recent research has broadened the spectrum of effects induced by HPV infection, showing that the E6 oncoprotein increases the

levels of reactive oxygen species and oxidative stress. This effect increases DNA instability and may be associated with chronic inflammation (Cruz-Gregorio et al. 2019).

Other studies have reported alterations in several representatives of the "rat sarcoma" (RAS) oncogene family in HNSCC. The HRAS isoform is mutated in 6% of HNSCC and associated with enhanced immune activity, with a high density of immune effectors in tumor samples (Lyu et al. 2019). The KRAS isoform is isolated in 17% of patients with HNSCC and associated with altered immune mechanisms, promoting immune tolerance via transforming growth factor-beta 1 (TGF-β1) (Weidhaas et al. 2017; Calenic et al. 2015).

Other genetic changes are independent of the HPV status. The activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), consequent to a loss of TP53, exerts pro-carcinogenetic effects by stimulating cell proliferation, angiogenesis, and epithelial to mesenchymal transition (EMT) (Aggarwal et al. 2006; Bharti and Aggarwal 2002). Experimental studies on HNSCC have revealed that a high intracellular concentration of active NF-κB is associated with increased metastatic capacity. NF-κB inhibitors are successful in decreasing cell invasive features by decreasing enzymes related to metastasis, such as vascular endothelial growth factor (VEGF) and matrix metallopeptidase 9 (MMP-9) (Yan et al. 2010). The activation of NF-κB has been detected in precancerous lesions frequently associated with HNSCC and in radio- and chemoresistant diseases. Deactivation of NF-κB in tumor cells leads to tumor regression in experimental models, triggering great interest in cancer therapy development.

While mandatory, genetic mutations are insufficient to develop cancer. The malignant cell requires appropriate conditions to multiply in an environment that provides protection from systemic tumor clearance mechanisms (Wellenstein and de Visser 2018).

3.2 Metabolic Changes in HNSCC

Frequent mitoses, characteristic of malignant cells, require high energy resources, implying an increased intracellular metabolism to cover the energy demand. Intense metabolic activity increases oxygen requirements, and tumor cells shift towards glycolysis to supplement energy production, leading to a decreased intracellular pH and accumulation of acidic byproducts. Intracellular acidosis seems an important element in local tumor progression and metastasis (Hosseini et al. 2017). Acidic pH of 6.5–6.9 is detected in the peritumoral environment. The theory of "acid-mediated tumor invasion" claims that a peritumoral acidic environment triggers necrosis in normal cells through pH-induced metabolic changes and activates MMPs that degrade the extracellular matrix, facilitating metastasis. Local acidosis exerts inhibitory effects on antitumoral immunity, promoting tumor progression. Various cancer experimental models show significant expression of markers for acidosis LAMP (lysosomal-associated membrane protein) and hypoxia (glucose transporter 1 and carbonic anhydrase IX (CA-IX)) (Ibrahim-Hashim and Estrella 2019). The CO_2 produced in tumoral metabolic conditions is eliminated from the cell via CA-IX activity, a membrane transporter that hydrates CO_2 and exports it as HCO_3^- and H^+ (Tafreshi et al. 2014). CA-IX activity is accelerated at pKa values under 6.5, modulating the acidity of a peritumoral environment. The inhibition of antitumoral immune surveillance is another consequence of acidosis, with alterations in cytolytic functions, a reduction of cytokine secretion, and the inhibition of signal transducers and activators of transcription 5/extracellular signal-regulated kinases (STAT5/ERK) signaling pathway (Calcinotto et al. 2012). In vivo experiments demonstrate that the use of oral buffer products correlates with increased efficiency of monoclonal antibody-based therapies (Pilon-Thomas et al. 2016).

Oxidative stress is closely correlated with carcinogenesis. Malignant cells show increased production of reactive oxygen species (ROS)

induced by exogenous or endogenous stimuli. ROS promote carcinogenesis through DNA instability. The subsequent mechanisms are tyrosine phosphatase inactivation and activation of the hypoxia-inducible factor 1 alpha (HIF-1α) pathway, which sustains malignant cell proliferation and immune cell anergy by further shifting cell metabolism to glycolysis (Vomund et al. 2017). In head and neck cancers, exposures to antioxidative agents, glutathione, and thioredoxin exert a strong inhibitory effect on cell proliferation and tumor growth (Roh et al. 2017). Multiple genetic alterations and metabolic changes are associated with tumor growth and metastasis in HNSCC. The effervescence of intracellular and local carcinogenic events generates a systemic response to counterbalance the progression to malignancy.

3.3 Inflammation in HNSCC

Chronic inflammation is an important element in carcinogenesis. Numerous studies show that indices of inflammation, assessed in the tumor tissue or peripheral blood, correlate with patient survival and response to therapy, leading to the evaluation of different inflammatory factors as biomarkers in HNSCC (Tampa et al. 2018b). Cytokines are small proteins released mainly by T helper cells and macrophages that play an essential role in regulating the immune response. Even though the immune cells are the primary source of cytokines, any nucleated cell carries the enzymes required for the synthesis and release of cytokines (Zhang and An 2007; Dinarello 2000). Pro-inflammatory interleukins have been intensively studied in the pathogenesis of HNSCC. The tumor milieu is characterized by chronic inflammation induced by various cellular and humoral mediated processes involving tumor, stromal, and immune cells. Increased concentrations of pro-inflammatory chemokines are reported in association with an inflammatory infiltration rich in macrophages, fibroblasts, and monocytes. Wang et al. (2002) have analyzed 86 tissue samples of HNSCC and demonstrated the overexpression of interleukin 6 (IL-6) and IL-6 messenger RNA compared to normal mucosa. This study also reveals a correlation between receptor expression for IL-6 and the tumor size and grading. IL-6 mRNA overexpression is associated with advanced disease and lymph and distant metastases. A study that assessed the immune gene expression in advanced HNSCC defined two out of the four tumor types with distinct immune profiles, presumably correlating with the HPV status. Pro-inflammatory cytokines and immune cell effectors were at high concentrations in the tumor microenvironment, defined as an enriched immune microenvironment (EIME), and correlated with prognosis (Cao et al. 2017). Sato et al. (2013) have suggested that determining salivary levels of IL-6 after treatment could act as a biomarker for early local and regional recurrence in HNSCC, while Jinno et al. (2015) have argued that IL-6 overexpression is associated with chemoresistant disease.

Significant correlations are also reported between cytokine levels and the progression of premalignant lesions to invasive malignant disease. Schiegnitz et al. (2018) have identified increased serum concentrations of IL-6, IL-8, and soluble IL-2 receptors in patients with HNSCC compared to patients who are either healthy or suffer from premalignant conditions, with IL-6 demonstrating a high statistical significance with large tumors and lymph node metastasis. The same study reports that patients with premalignant lesions have higher levels of IL-8 than controls. Salivary levels of IL-8 have been analyzed in patients suffering from premalignant lesions and oral squamous cell carcinoma compared to healthy subjects. The results demonstrate that only subjects with invasive lesions show significantly higher concentrations of IL-8, thus recommending salivary IL-8 as a potential biomarker for the diagnosis and follow-up of oral squamous cell carcinoma, but not of premalignant lesions (Punyani and Sathawane 2013). The NF-κB pathway discussed above might be the underlying mechanism for IL-8-mediated HNSCC tumorigenesis, confirming the role of chronic inflammation in malignant transformation (Rao et al. 2010).

Interactions between inflammation and carcinogenesis described in oral squamous cell carcinoma have also been identified in cutaneous squamous cell carcinoma (Scheau et al. 2020). In UV-induced skin and lip cancers, activation of intracellular NF-κB signaling pathways triggers a cascade of inflammatory events, including the secretion of IL-8 by macrophages and monocytes, which eventually leads to tumor progression (Neagu et al. 2019; Balkwill and Coussens 2004). The involvement of chronic inflammation, fueled by the release of cytokines, underlies malignant transformation, local invasion, and metastases. Conversely, inhibition of the immune response by anti-inflammatory agents correlates with a decrease in UV-induced cancer incidence (Wright et al. 2006).

The anti-inflammatory cytokines IL-10 and IL-13 have been found significantly increased in saliva of HNSCC patients and tumor tissue of oral squamous cell carcinoma (Aziz et al. 2015; Chen et al. 2013). The increase correlates with poor prognosis, especially in early disease, suggesting that a strong immunosuppressive environment at the initial stages is conducive to aggressive tumor cell behavior, promoting tumor growth and progression.

3.4 Assessment of Inflammation in HNSCC in Clinical Setting

The detection of sophisticated elements for cancer diagnosis in early stages and disease follow-up requires expensive laboratory equipment, trained staff, expensive kits, which may be available in a research unit, but are difficult to access in the clinical setting for general use. The current clinical practice evaluates inflammation through cellular and biochemical markers of the systemic immune response, and several studies have reported results that support the prognostic value of markers in various cancers.

Circulating proteins involved in systemic inflammation have been studied as potential prognostic biomarkers in different malignancies. C reactive protein (CRP) increases in inflammation, infection, and trauma. Recent data show a strong correlation between the CRP level and cancer. A study that followed a large group of subjects from the general population for 16 years has reported an increased rate of malignancies in patients with a high serum CRP content. Additionally, increased baseline CRP in cancer patients was associated with poor prognosis (Allin et al. 2009). The onset of cancer correlates with sustained inflammatory status induced by the intense metabolic reactions in tumor tissue. This systemic inflammatory process, translated in increased serum content of CRP, among other changes, is described in cancer patients (Asegaonkar et al. 2015). In OSCC, higher values of CRP are detected in patients exposed to risk factors (smoking and alcohol) and correlate with an increased rate of regional progression through lymph node involvement and poor prognosis (Tai et al. 2017). Similar results in HNSCC are reported in several studies, correlating an aggressive tumor behavior with unfavorable prognosis and elevated levels of CRP (Peter et al. 2013; Khandavilli et al. 2009). Some authors studied CRP in relation to other parameters. Glasgow prognostic score and its refined variants include serum albumin, alongside CRP, and are used in stratifying the clinical risk of patients with various cancers (Pan et al. 2017; Saijo et al. 2017). These scores have been analyzed in patients with operable HNSCC and reported data to support their prognostic potential (Hanai et al. 2018; Selzer et al. 2016; Farhan-Alanie et al. 2015).

Serum fibrinogen correlates with disease progression in different cancers (Zhang et al. 2020; Xu et al. 2018). This glycoprotein, known for its role in coagulation, is also involved in the cancer-associated systemic inflammatory response, and elevated levels of fibrinogen correlate with poor prognosis in many malignancies (Grafetstätter et al. 2019). In solid tumors, progression to malignancy and local invasiveness correlate with changes in structural components present in the extracellular environment. Deposits of fibrinogen and fibrin have been identified in tumor stroma promoting fibroblast proliferation and angiogenesis mediated by overexpression of IL-1, VEGF (vascular endothelial growth factor), and FGF-2 (fibroblast growth factor 2) (Mosesson 2005;

Simpson-Haidaris and Rybarczyk 2001). The link between fibrinogen and cancer progression is still unclear. However, various studies reveal strong correlations with prognosis in patients with HNSCC. In locally advanced OSCC, high pretreatment fibrinogen values have been detected in patients with resistance to radio- and chemotherapy and correlated with disease recurrence and poor survival (Holzinger et al. 2016). Other studies have shown similar results in HNSCC, where a high serum fibrinogen content correlates with advanced disease, presence of metastases, and unfavorable prognosis (Yang et al. 2019; Lan et al. 2016).

4 Immune Landscape in Carcinogenesis

4.1 Defensive Mechanisms Against Cancer

The role of the immune system in carcinogenesis was suspected more than a century ago by W.B. Coley, a surgeon who reported a series of cases treated by bacterial inoculation in tumor mass. The therapy relied on triggering a local immune response that would destroy tumor cells (Coley 1910). The important role of the immune system in the protection against cancer was confirmed by the substantial increase in the incidence of cancers, especially viral related, in subjects with immunodeficiency syndromes of various causes (Vajdic and Van Leeuwen 2009).

In the complex antitumor defense machinery, intrinsic cellular mechanisms are doubled by extrinsic protection systems in which immune cells are key players. While intrinsic tumor suppression mechanisms are active and efficient, the immune response is not initiated. The accumulation of genetic alterations, such as mutations of the p53 suppressor gene, leads to an increased intracellular concentration of p53 peptide, shifting the metabolic pattern of the affected cell. Subsequently, opposing intracellular mechanisms are overcome and the mutant cell is beyond repair, entering a stabilization phase called cellular senescence, which implies changes in its secretory phenotype (Kuilman et al. 2010). It initiates the release of pro-inflammatory cytokines generating an immune reaction guided to destroy the senescent cell, thus preventing the progression to malignancy (Kuilman et al. 2008). An increased density of cells with the senescence-associated secretory phenotype (SASP) is reported in precancerous lesions of OSCC, which may act as the first step in cancer prevention by inducing a lymphocyte T CD4+ mediated immune response (Johnson et al. 2016; Campo-Trapero et al. 2008). Progressive mutations with p53 loss of function together with other gene alterations push mutant cells out of SASP status leading to the development of invasive features (Wellenstein and de Visser 2018). In this stage of carcinogenesis, if the balance favors the accumulation of mutations and overruns the clearance capacity of the immune system, malignant cells survive, setting the debut of cancer. In established malignancies, lymphocytes T CD8+ are the main antitumor effectors. Little mobilization of these cells in premalignant lesions could be a prevention mechanism of autoimmunity mediated by cytotoxic T lymphocytes (Ostroumov et al. 2018). Studies conducted on murine models show that CD4+ T lymphocytes that release interferon-gamma (IFN-γ) and TNF-α can reactivate the SASP mechanisms and induce growth arrest in tumor cells, promoting immune clearance of malignant cells, independently from intracellular protection mechanisms (Braumüller et al. 2013).

Three steps have been defined in the immune antitumor defense process: elimination, equilibrium, and escape (see Fig. 2). Throughout these steps, immune cells undergo tumor immunoediting, a process in which highly immunogenic cells with an intense expression of tumor-specific antigens (TSA) are removed. In the experimental setting, tumor cells generated in immunodeficient animal models do not progress to malignancy after transplantation into immunocompetent hosts, because the immunoediting and proliferation of poorly immunogenic cell lines do not take place (Smyth et al. 2006). In the initial elimination phase, newly created tumor cells are efficiently removed by the immune system (Mittal

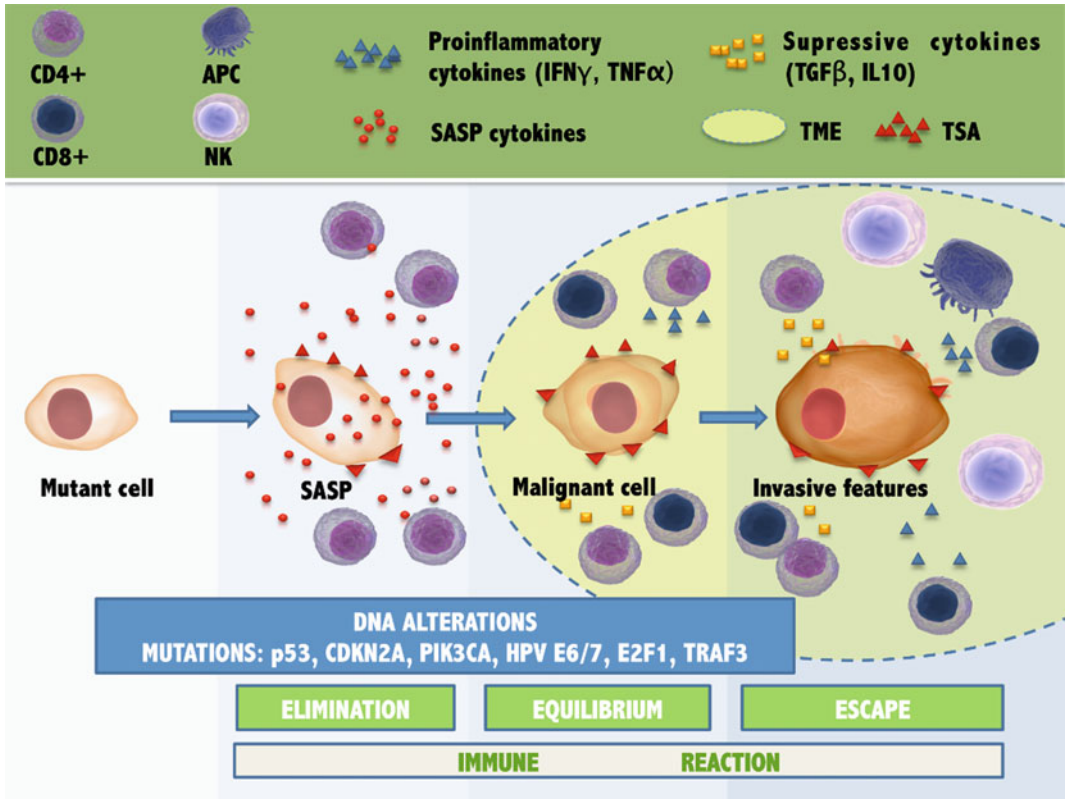

Fig. 2 Graphic representation of genetic and immune dysfunctions leading to carcinogenesis. Progressive accumulation of intracellular genetic alterations leads to a switch to senescence-associated secretory phenotype (SASP) with specific cytokine release that initiates activation of the immune response, mainly mediated by CD4+ lymphocytes. Intensely immunogenic cells, expressing tumor-specific antigens (TSA) are eliminated, leading to the selection of mutant clones with decreased immunogenicity in the equilibrium phase. Tumor microenvironment (TME) formation is initiated with modulatory effects on immune effectors promoting progression to cancer with invasive features. In the escape phase, progressive accumulation of immune cells: CD8+ lymphocytes, natural killer cells (NK), antigen-presenting cells (APC) together with CD4+ lymphocytes can be detected, with different, often antagonistic, effects on tumor growth

et al. 2014). This may explain the spontaneous tumor remission described in clinically manifest malignancies (Markowska and Markowska 1998). Overcoming the capacity of immune clearance of malignant cells pushes forward carcinogenesis into the equilibrium phase, where tumor progression is controlled by immune surveillance mechanisms. Malignant cells will not be fully eliminated, and by adding new mutations, they will generate cellular clones with decreased immunogenicity. The tumor microenvironment is initiated in this stage of carcinogenesis, which will promote survival of malignant cells and finally advancement to the escape phase of the immune response, favoring cancer progression (Bottomley et al. 2019). Equilibrium phase mechanisms might explain the pathogenesis of occult malignancies, frequently encountered in HNSCC, where the immune system counteracts local tumor development (Koebel et al. 2007). The clinical manifestation of cancer occurs when tumor cells have survived the intrinsic antitumor control mechanisms, the senescent cell elimination phase, and have passed immunoediting processes. However, tumor cells never achieve a complete immunotolerance state.

When the immune cells, collected from the tumor microenvironment and processed in vitro, are transferred to lymphopenic mouse models with malignant melanoma, they induce tumor regression (Quezada et al. 2010; Dudley et al. 2008). The same results are reported after the autologous transfer of genetically engineered lymphocytes (Morgan et al. 2006). Ribatti (2017) labels this phenomenon with the term "tumor immunosurveillance". Tumor cell immunoediting and immune escaping are defined as fundamental elements of carcinogenesis (Hanahan and Weinberg 2011).

4.2 Tumor Microenvironment

The tumor microenvironment (TME) is an independently studied entity in cancer pathogenesis. The cellular component in TME is represented by tumor cells and non-tumor elements: stromal cells and immune cells, playing a major role in carcinogenesis through their complex interactions. Cancer pathogenesis should not be simplistically regarded as a sum of tumor cell features but rather as a distinct structure with independent pathophysiological characteristics. Inflammatory tumor infiltrations are present in all cancers, with variable intensity, ranging from patterns undetectable through standard evaluation techniques to intense inflammatory types of cancer. The complex interactions between stromal components of TME, inflammatory, and tumor cells are not yet completely unveiled (Thorsson et al. 2018; Cai and Jin 2017).

Tumor stromal acellular composition can vary from a high content of loose connective tissue to an increased number of collagen structures that grant a fibrotic aspect to the lesion. These stromal elements can organize tumor cell nests (TCN) of variable size, with presumed prognostic characteristics in several malignancies (Alsibai and Meseure 2018; van Pelt et al. 2018). A recent study has analyzed several specific tumor stroma parameters, such as stroma-tumor ratio (TSR), stroma type, and TCN size in patients with HNSCC, showing that the increased TSR, defined by a fibrotic stroma with frequent small-sized TCNs, is indicative for poor prognosis contrarily to the large TCN and low TSR, which correlate with a better response to chemotherapy (Karpathiou et al. 2019).

4.3 Immune Cells in HNSCC

The TME hosts various subtypes of differentiated immune cells, with contrasting effects on tumorigenesis and myeloid progenitors that exhibit carcinogenic activity (Hanahan and Weinberg 2011). T-cell tumor infiltrations and their interactions within TME have been rigorously studied, yet the complex mechanisms related to tumor progression, prognosis, and resistance to immune therapy are far from being uncovered (Anderson et al. 2017).

Studies have correlated local immune reaction with TMB of malignant cells. Malignancies exhibit diversity in cell mutational burden, the highest rate of mutations being identified in chronic exposure to risk factors that induce DNA alterations, such as UV exposure or smoking. In consequence, UV and nicotine-induced squamous cell carcinomas have been reported to possess one of the highest mutational burdens (Fancello et al. 2019). Tumors with a high TMB are characterized by an increased expression of tumor-specific antigens (Rooney et al. 2015). These antigens result from degrading self-proteins into 8–10 amino acid-long peptides that activate T-cells, triggering an antitumor immune reaction (Antunes et al. 2018). Both innate and adaptive immune systems are directed towards eliminating malignant cells, through mechanisms mediated by major histocompatibility complex (MHC), implying TSA-mediated activation of immune effectors. Except for viral-induced malignancies (HPV or EBV), tumor cells derived from self-structures expressing MHC Class I should not trigger an immune response. However, TSAs are presented mainly through MHC class I and can be recognized by the immune effectors (Schumacher et al. 2019). Decreased expression of MHC Class I molecules on tumor cells is another mechanism of immune escape. However, malignant cells with negative MHC Class I status

are eliminated by NK cells (Garrido 2019). NK cells show an increased affinity for cancers with low or lacking MHC Class I expression, called "missing self" cells, by recognizing the absence of normal elements on the cell surface known as "absence of the expected" (Kärre 2008). This phenomenon is used on developing molecules that promote NK-mediated tumor cell clearance, either independently or in association with T lymphocytes, especially in MHC Class I-negative cancers (Minetto et al. 2019). In HPV-positive HNSCC, MHC Class II plays an important role. Viral antigens bind to MHC Class II molecules that are overexpressed on tumor cells, acting as antigen-presenting cells. An intense immune response is generated that is associated with a significantly improved prognosis (Gameiro et al. 2019). However, overexpression of TSAs is present only on a subpopulation of tumor cells, triggering an ineffective immune response that may explain resistance to targeted therapies (McGranahan et al. 2016). Based on these findings, some cancers, including HNSCC, are divided into subtypes according to immune and genetic features that correlate with prognosis and response to therapy (Cao et al. 2017; Keck et al. 2015).

Tumor-infiltrating lymphocytes (TILs) show high variability in cancers. Cytotoxic T-cells (CD8+), helper T-cells (CD4+), regulatory T-cells (Treg), and B lymphocytes coexist in TME with the leucocytes of the innate immune family, such as NK cells, innate lymphoid cells (ILC), macrophages, neutrophils, monocytes, antigen-presenting cells (APC), and dendritic cells, exerting synergic or antagonistic effects.

Cytotoxic T-Cells (CD8+) The main effectors of adaptive immune responses, when present in large numbers in TME, predict a better prognosis in most cancers (Mazzaschi et al. 2018; Li et al. 2017). Similar results have been reported for patients with HNSCC, where the density and distribution of CD8+ lymphocytes in TME seem to have independent prognostic capabilities (see Table 1). A study conducted on 139 patients with oral squamous cell carcinoma has reported that high numbers of CD8+ T-cells in parenchyma around the invasion front and stromal CD8+ cells at the tumor periphery independently correlated with improved survival and low recurrence rates (Shimizu et al. 2019). HPV status in HNSCC plays an important role in the immune infiltration of TME. Thus, HPV-positive tumors show a higher CD8+ T-cell infiltration compared to HPV-negative ones in oropharyngeal squamous cell carcinoma, which also correlates with better prognosis when CD8+ T-cells concentrate in stromal areas (Oguejiofor et al. 2015). This finding most likely relies on the synergic immune recruitment mechanisms mediated by two distinct triggers, TSA and viral antigens, which intensify the local immune reactivity. Näsman et al. (2012) have reported similar results in tonsillar SCC in that patients with HPV-positive tumors display a higher number of CD8+ T-cells compared to HPV-negative ones, and an enriched CD8+ T-cell environment correlates with better survival. Another study conducted on 270 subjects with oropharyngeal squamous cell carcinoma has identified a small subgroup of patients with HPV-positive tumors that exhibited low concentrations of tumor-infiltrating lymphocytes (TILs), with a prognosis similar to HPV-negative tumors (Ward et al. 2014). CD8+ T-cells rely on interaction with CD4+ T lymphocytes to exert their cytotoxic functions.

Helper T Lymphocytes (CD4+) They initiate and maintain antitumor immune reaction by mobilizing CD8+ T-cells that recognize specific tumor antigens presented by MHC molecules and trigger tumor cell death (Ostroumov et al. 2018). T helper cells are involved in all aspects of antitumor immunity, including interaction and mobilization of innate immune system representatives. Different antigen-presenting cells migrate to draining lymph nodes and induce T-cell activation mediated by TSAs, generating T-lymphocyte clones with antitumor specificity that will perform their antitumor effects mainly at the tumor site. This process is called the tumor immune cycle (Chen and Mellman 2013). The role of CD4+ is controversial as these cells are defined as "double-edged swords" due to their opposing effects in cancer (Das et al. 2018). Naïve CD4+ T-cells undergo differentiation to

Table 1 Cytotoxic T cells in the pathogenesis of HNSCC

Density (TIL score)	Distribution compartment	Survival improvement	Decrease in recurrence rate	HPV infection status	Study type	References
High	Peripheral	Yes	Yes	N/A	139 cases OSCC	Shimizu et al. (2019)
High	N/A	Yes	N/A	Positive	83 cases TSCC	Näsman et al. (2012)
High	Tumor/stroma	Yes	Yes	Positive	270 cases OPSCC	Ward et al. (2014)
High	Stromal	Yes	Yes	Positive	218 patients OPSCC	Oguejiofor et al. (2017)
High	All	Yes[a]	N/A	Positive	203 cases HNSCC	Ngamphaiboon et al. (2019)

TIL Tumor-infiltrating lymphocyte score, *HPV* Human papillomavirus, *TSCC* Tonsillar squamous cell carcinoma, *OSCC* Oral squamous cell carcinoma, *OPSCC* Oropharyngeal squamous cell carcinoma, *HNSCC* Head and neck squamous cell carcinoma

[a]Concerns non-OPSCC only

activated T helper 1 populations (Th1) in an environment rich in IL-12 or T helper 2 cells (Th2) in the case of a high IL-4 concentration and the absence of IL-12 (Knutson and Disis 2005). Th1 are major antitumor effectors due to cytokine secretion and activation of tumor cell receptors that initiate cell death. Th1 cells act via IFN-γ and TNF-α to induce cancer cells into senescence and initiate their apoptosis (Braumüller et al. 2013). They regulate cytotoxic immune responses and contribute to tumor suppression. Increased expression of specific Th1 cell genetic markers in TME correlates with a good prognosis (Bindea et al. 2013). Some studies suggest that CD4+ T-cells can eliminate tumor cells, in the absence of CD8+ T-cell-mediated immunity, but the cooperation of both cell lines increases the efficiency of antitumor immunity (Fukunaga et al. 2004). In the case of HNSCC, data are not conclusive and often inconsistent regarding CD4+ T-cells. A study assessing the infiltration of CD8+ and CD4+ T-cells in HNSCC has failed to find any relationship of CD4+ T-cells with survival or the HPV status (Nordfors et al. 2013). Different results have been reported in another study where patients exhibiting a high density of activated CD4+ T-cells had better survival and improved local control (Badoual et al. 2006). A meta-analysis assessing prognostic correlations of different types of TILs in HNSCC has reported favorable outcomes in tumors with high TILs for both CD3+ and CD8+ T-cells but failed to find significant correlations for CD4+ T-cells (de Ruiter et al. 2017). The difficulty in assessing prognostic properties of CD4+ T-cells probably has to do with the subgroups of cells that have antagonistic effects in cancer. Regulatory T-cells (Treg Foxp3+) have been intensively studied and are suspected to promote tumorigenesis through immunosuppressive effects. In normal conditions, Tregs, marked with CD25+ and transcription factor Foxp3+, are responsible for maintaining immune tolerance and autoimmunity prevention (Sakaguchi 2005). Increased expression of Treg markers in TME and peripheral circulation is often associated with tumor progression. Tregs show inhibitory effects on CD4+, CD8+, and dendritic cells, inducing immune cell exhaustion through cytokine release, like TGF-β or IL-10 (Bauer et al. 2014; Jarnicki et al. 2006).

In some cancers, TME displays an immunosuppressive effect on CD4+ T-cells by stimulating their differentiation into the Treg subtype (Zheng et al. 2009). In cutaneous SCC, disease aggressiveness correlates with a high density of Treg cells and increased concentrations of IL-10 and TGF-β (Azzimonti et al. 2015). However, contrasting results have been reported for HNSCC where tumor infiltration with FoxP3+

T-cells is identified in patients with improved prognosis (de Ruiter et al. 2017). A similar favorable prognostic correlation for intense Treg infiltration has been reported in colorectal cancers (Salama et al. 2009). The innate immune system has been widely studied in cancers, along with adaptive immunity.

Natural Killer (NK) Cells These cells are the main representatives of innate immunity and are considered equivalents of cytotoxic T-cells in the adaptive immune system. NK cells trigger death in infected or cancer cells via the release of enzymes, perforins, and cytokines, like TNF family members (Spits et al. 2016). Tumor cells have protection mechanisms against innate immunity that rely on TGF-β release with alterations in cytotoxic abilities of NK cells (Cortez et al. 2017). Immature NK cells (CD3-CD56brightCD16-) are activated by cytokines and IFN-γ. Mature NK cells (CD3-CD56dimCD16+) release cytoplasmic inclusions with enzymes and perforins that act directly on malignant cells inducing their lysis. CD16+ receptor expressed on NK cells binds with tumor cells through IgG molecules initiating their activation. Co-activatory stimuli, such as IL-2, IL-12, IL-15, IL-18, and IFN-γ are necessary to induce NK cell cytotoxic function leading to malignant cell clearance (Hu et al. 2019; Minetto et al. 2019). These molecules can be detected in TME. High concentrations of activated NK cells as a complementary defense system, alongside immunity mediated by T and B lymphocytes, has been associated with better outcomes (Nair and Dhodapkar 2017). Tumor cells use protective mechanisms against being recognized by NK cells. Denaturation of peptide constituents from specific ligands affects NK receptor interaction, altering the NK-mediated immune defense (de Andrade et al. 2018).

Lymphocytes B There is a debate regarding the role of lymphocyte B infiltrates in cancers. Studies in experimental models have shown that in genetically modified mice with depletion of lymphocytes B, there is no progression to epithelial malignancies (de Visser et al. 2005). In HNSCC, a correlation has been reported between the HPV status and B cell population, with prognostic potential. Patients with an intense lymphocyte B infiltrate alongside CD8+ cells have a significantly improved prognosis. It is suggested that activated B cells release the chemokine (C-X-C motif) ligand 9 (CXCL9) cytokine that supports the recruitment of CD8+ lymphocytes, promoting tumor cell clearance (Hladíková et al. 2019). The role of B cells in HNSCC pathogenesis has been emphasized in another study that reports high intra-tumoral percentages of antigen-presenting, activated, and memory B cells (Lechner et al. 2019). These findings encourage further research and support the potential role of B cells as new therapeutic targets in HNSCC.

Tumor-Associated Macrophages (TAM) High densities of tumor-associated macrophages (TAMs) were identified in many cancers, including epithelial malignancies, acting as tumor-promoting agents (Li et al. 2020). TAMs release VEGF and MMP, facilitating angiogenesis, local invasion, and metastasis (Evrard et al. 2019). In advanced HNSCC, in patients with enhanced immune profile with associating high densities of TAM, signaling for immune checkpoint ligands is upregulated, which also correlates with poor prognosis (Cao et al. 2017). A recent meta-analysis has reported that a high density of TAMs in HNSCC is associated with advanced tumors, nodal staging, and vascular and lymphatic invasion (Kumar et al. 2019). Refinement of TAM detection, which seems of prognostic significance, is suggested in another meta-analysis. Immunosuppressive subtype M2 of TAMs (CD163+) strongly correlates with a worse prognosis in patients with HNSCC (Troiano et al. 2019).

Antigen-Presenting Cells (APC) These cells play an important role in carcinogenesis. TSAs are presented through the MHC, initiating an adaptive immune response with the generation of antigen-specific T-cell clones (Bottomley et al. 2019). In HNSCC, dendritic cells (DCs) act as APCs together with lymphocytes B, TAMs, and other immune cells (Wondergem et al. 2020). There are contradictory data concerning DCs and their influence on the

prognosis in HNSCC, with some studies reporting a favorable outcome in association with a high density of DCs (Jardim et al. 2018) while others report opposing results (Hilly et al. 2016).

4.4 Immune Checkpoints in HNSCC

The complexity and diversity of TME and the multitude of interactions between all its elements make it hard for the scientific community to unveil many of its mysteries. A recent discovery of immune checkpoint molecules has revolutionized the world of immunology and pathophysiology in cancer and enriched the pallet of cancer therapies with an innovative approach through immune checkpoint inhibitors.

In advanced HNSCC, an enhanced immune cell infiltrate often exhibited overexpression of checkpoint molecules. These molecules, which normally act as a protection mechanism against excessive immune reactions and autoimmunity, have a negative influence on cancers. Signaling pathways initiated through the checkpoint molecules weaken the reactivity of T-cells and induce an anergic status known as "immune exhaustion". It can affect both CD4+ and CD8+ T-cells and is reversible under the action of checkpoint blockade therapies, rendering back function to exhausted immune cells (Ostroumov et al. 2018).

Cytotoxic T lymphocyte antigen 4 (CTLA-4), expressed mainly by T-cells, is a transmembrane protein with affinity for B7 molecules, expressed on APCs. B7 molecules, represented by two subtypes CD80 and CD86, bind with CD28 expressed on T-cells, with costimulatory action in addition to TCR activation (Carreno and Collins 2002). The binding of CD80/CD86 to the costimulatory receptor CD28 activates T lymphocytes with simultaneous overexpression of CTLA4 (Mei et al. 2020). Competitive binding of CTLA4 to B7 molecules, due to a higher affinity of CTLA4 for CD80/CD86 rather than for CD28, induces T-cell exhaustion and an overall effect of immunosuppression (Postow et al. 2015).

Programmed cell death protein-1 (PD-1), expressed on activated immune cells, has a negative regulatory effect on T-cells, after binding to its specific ligands, PD-L1 and PD-L2, present on tumor and immune cells (Mei et al. 2020). This leads to an intracellular signaling blockade that interferes with normal activation and proliferation of T-cells (Alsaab et al. 2017). However, studies have shown that in different malignancies, through repeated mutations, clones of immune cells resistant to PD-1 blockade are selected (Hugo et al. 2016; Koyama et al. 2016). Expression of PD-1 and PD-L1 in HNSCC is influenced by the inflammatory cytokines IFN-γ and TNF-α and shows high dynamics, which can explain resistance to immunotherapy (Oguejiofor et al. 2017). PD-1 and PD-L1 are also considered the key players in the initiation and progression of HPV-induced HNSCC (Lyford-Pike et al. 2013). The prognostic value of PD-L1 has been suggested in a study on 203 patients with HNSCC showing a high infiltration with CD8+ in correlation with improved overall survival. However, patients with moderate and high expression of PD-L1 have a worse prognosis compared to those with low PD-L1 expression, defined as less than 1% (Ngamphaiboon et al. 2019).

Immune exhaustion affecting CD4+ T-cells features an overexpression of inhibiting coreceptors, like T-cell immunoglobulin mucin-3 (TIM-3). The binding of TIM-3 with the specific ligand galactin-9 induces CD4+ T-cell apoptosis and a functional deficit in CD8+ T-cells (Mei et al. 2020). Studies in experimental models have reported an increased tumor recurrence rate associated with immune exhaustion of CD4+ T-cells, suggesting that combined immune therapies would be more effective in controlling recurrent malignancies (Koyama et al. 2016). Exhausted immune cells are characterized by alteration of proliferation, cytokine secretion, and deficient recognition of specific antigens. The clinical expression of immune exhaustion is tumor progression and recurrence (Xia et al.

2019). The heterogenic character of HNSCC raises challenges in immunotherapy research due mainly to etiological and immunological differences that require an individualized approach for each patient (Perri et al. 2020).

5 Biomarkers in the Management of HNSCC

The determination of immune biomarkers may significantly influence the management of cancer patients. The prognostic feature of biomarkers in cancers could allow the stratification of patients into risk groups, facilitating early identification of patients with resistance to therapy or aggressive disease. Also, the potential of repeated measurements when analyzing circulating biomarkers assists the patient monitoring and provides early notification of tumor progression. Unraveling the mechanisms of antitumor immunity is one of the steps in the identification of new therapeutic targets that may guide and modulate the antitumor immune response.

In HNSCC, as in many cancers, biomarkers are an emerging subject of interest. Studies have revealed a large number of molecules that promise to predict prognosis, resistance to therapy, or recurrence (Tampa et al. 2018b). Our research group has extensively studied neuroendocrine factors as potential HNSCC biomarkers that can further clarify the pathogenesis and disease progression (Solomon et al. 2018; Lupu et al. 2017). Currently, prognosis in HNSCC is mainly dictated by clinical staging and primary location. Therefore, 5-year survival rates can be higher than 90% in the early stages of lower lip tumors and drop below 40% in advanced oral tumors with distant metastasis (Hladíková et al. 2019). The tumor HPV status is another marker with confirmed prognostic potential in many studies (Kobayashi et al. 2018, Chakravarthy et al. 2016), although one study identified a subset of HPV-positive tumors that did not differ in terms of prognosis from HPV-negative disease (Ward et al. 2014). Local and systemic antitumor immunity has been intensively assessed as biomarkers in HNSCC. Tumor-infiltrating lymphocytes (TILs) have been identified as prognostic markers independent of HPV status, and an increased density of TILs and CD8+ T-cells correlates with higher survival rates (Heikkinen et al. 2019; de Ruiter et al. 2017; Distel et al. 2009). A quick semiquantitative method for evaluating TILs, called TILws (weight sum score), facilitates their use as prognostic biomarkers in the clinical setting (Spector et al. 2019).

6 Emerging Therapies for HNSCC Patients

The increased interest for antitumor immunity has sparked, following the observation that the stimulation of immune elements caused tumor regression even in advanced disease stages. Therefore, immune system modulation has been intensively investigated and became a part of the anticancer arsenal, with outstanding results in some types of neoplasia (Dobosz and Dzieciątkowski 2019; Trapani and Darcy 2017).

Standard treatment in HNSCC consists of surgery, radiotherapy, or a combination of the two and is guided by disease staging and anatomical location, disregarding biological characteristics of the tumor. Early stages (I and II) may be treated by surgical resection or radiotherapy, with similar results in disease control. Combined therapy is recommended in advanced stage II and stage III cases. Standard therapy includes resection of cervical lymph nodes or extended neck radiotherapy (National Cancer Institute 2015). Neoadjuvant chemotherapy is investigated in locally advanced diseases and the TPF regimen (docetaxel, cisplatin, and fluorouracil) improves local disease control and prognosis after surgery (Haddad et al. 2018). A 4% increase in the survival rate has been reported when chemo- and radiotherapy are combined in unresectable advanced disease (Pignon et al. 2001).

Immunotherapy and target therapy for advanced and metastatic HNSCC have been recently approved. These therapies mediate the activation or reactivation of the immune system by modulating its antitumor effects. The first targeted therapy approved by the Food and Drug

Administration (FDA) and the European Medicines Agency for the treatment of recurrent and metastatic disease was cetuximab, an inhibitor of epidermal growth factor receptors (EGFR). Cetuximab added to chemotherapy increases the response rate to treatment by 13% in HNSCC (Vermorken et al. 2008). In 2019, the same regulatory institutions approved the use of anti-PD1 monoclonal antibodies, nivolumab and pembrolizumab, for treatment of recurrent and metastatic disease in chemoresistant patients. These agents block PD-1 receptors overexpressed on T-cells and prevent specific ligand binding, such as PD-L1 and PD-L2 expressed by tumor cells, which exert inhibitory effects on immune cells (Xia et al. 2019). Pembrolizumab is approved as the first-line therapy in association with chemotherapy in patients with metastatic or unresectable disease but can also be administered alone in tumors with PD-L1 overexpression, with a combined positive score of ≥ 1 (Cohen et al. 2019a). Other therapeutic regimens of monoclonal antibodies are under review for advanced HNSCC. For instance, a human monoclonal antibody against CTLA-4, tremelimumab, prevents binding of the inhibiting receptor CTLA4 expressed on immune cells with the specific ligands CD86 and CD80. CTLA4 blockers act on Treg cells inducing their depletion and increasing the ratio of effector-to-Treg cells (Selby et al. 2013). Phase III study EAGLE has evaluated the association between a new anti-PD1 agent, durvalumab, and tremelimumab in patients with metastatic and recurrent disease, reporting no significant differences in survival rates compared to the group receiving cetuximab and chemotherapy (Ferris et al. 2020). Molecules that stimulate NK-mediated immunity are also studied in patients with HNSCC as they are the main effectors in antitumor defense, alongside CD8+ T-cells. Monalizumab is the first member of this class that acts on the natural killer cell receptor (NKG2A) expressed by CD8+ and NK cells present in TME. Preliminary results from an ongoing study investigating the association of monalizumab and cetuximab in 40 patients with recurrent or metastatic disease are encouraging (Cohen et al. 2019b). NK-activating interleukins are also studied as potential therapeutic agents in HNSCC. Infusion of IL-2 in experimental models led to severe adverse reactions and an exponential increase of Tregs inducing antitumor tolerance, invalidating any potential benefits in repelling cancer (Nelson 2004). Infusion of NK cells has been experimentally attempted, and when associated with IL-15 it determined complete tumor regression (Miller et al. 2005). Treg expansion induced by IL-2 might be overcome through the administration of IL-15. Preclinical studies have shown that IL-15 can activate NK and CD8+ T-cells without inducing Treg expansion (Waldmann 2015). Studies evaluating the effects of IL-15 administration have reported a significant increase in NK cells and, secondary, in CD8 + T-cells that additionally contribute to increasing NK cell density through a CD16-mediated pathway (Miller and Lanier 2019; Nair and Dhodapkar 2017).

HNSCC prevention is also of major interest, especially considering the increased incidence of HPV-related disease. As global anti-smoking campaigns induced a decrease in smoking-related HNSCC (Rettig and D'Souza 2015), anti-HPV vaccination is anticipated to yield similar results. A series of vaccines targeting HPV-positive HNSCCs is under testing, and preliminary results support their role in preventing the progression of dysplasia to cancer (Wang et al. 2018). Intratumor injection of viral agents is also investigated in advanced HNSCC (Old et al. 2016), after good results in the treatment of malignant melanoma have been reported, which led to FDA approval of oncolytic virus, talimogene, in this malignancy (Bommareddy et al. 2017). HNSCC is accessible for this type of therapy, and the reported effects on circulating T-cells in patients with solid tumors that do not respond to treatment support the role of viral therapy in fighting cancer by stimulating the antitumor immune response (Taipale et al. 2015). Thus, there are outstanding prospects for preventing and curing oncological ailments. HNSCC is regarded with a great interest

for emerging therapies that will advance the management of this disease.

7 Conclusions

Major improvements in disease control and survival in HNSCC have been correlated with staging. Thus, early diagnosis is of paramount importance to detect patients at high risk of an unfavorable outcome, allowing the appropriate therapeutic approach. Numerous studies have shown that inflammation is a key player in HNSCC progression, regardless of cancer location and the main risk factors involved. Moreover, immunity has been highlighted as a major factor in disease control. Further studies in this area of research will allow unveiling new mechanisms in the complex connections between immuno-inflammatory processes and carcinogenesis and will open new possibilities to identify more reliable and widely applicable predictive factors and therapeutic targets in HNSCC.

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Outcome of Repeat Pulmonary Metastasectomy

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Abstract

Pulmonary metastasectomy is a well-established contribution to the cure of oligometastatic cancers, but its exact effectiveness is poorly understood. Here we report the outcomes of repeat pulmonary metastasectomy from a multicenter trial. This retrospective study included patients who underwent re-do metastasectomies between January 2010 and December 2014. The exclusion criterion was metastasectomy without curative intent. We reviewed medical files of 621 consecutive patients who underwent initial pulmonary metastasectomy. Of those, 64 patients underwent repeat metastasectomies, and these patients were included in the analysis. All the 64 patients underwent a second metastasectomy, later 35 of them underwent a third metastasectomy, 12 underwent a fourth metastasectomy, and 6 underwent a fifth metastasectomy. The total number of re-do

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metastasectomies was 181. The median overall survival among the patients undergoing re-do metastasectomy was 66.0 ± 3.8 months. Three and 5-year survival rates were 82.3% and 63.3%, respectively. The 5-year survival rates were 63.3% after the first, 50.9% after the second, 74.4% after the third, 83.3% after the fourth, and 60.0% after the fifth metastasectomy. We conclude that at the current stage of knowledge, there is an indication for repeat re-do metastasectomy with curative intent.

Keywords

Cancer · Lung metastases · Metastasectomy · Pulmonary metastasectomy · Survival

1 Introduction

Pulmonary metastasectomy represents a key therapy option in the treatment of lung metastases, enhancing the chance of long-term survival for selected patients with oligometastatic cancer. Several studies have investigated the benefits of repeated metastasectomy (Yang et al. 2020; Matsumoto et al. 2019; Menna et al. 2018; Salah et al. 2013; Borasio et al. 2011; Welter et al. 2007). Unfortunately, these studies are marred by small numbers of patients, the inclusion of patients from previous eras, or a short follow-up. Mineo et al. (2015) have demonstrated good results following the first re-do metastasectomy, but a less favorable outcome after the second or third re-do procedure. However, that study included patients from the years 1986 to 2010. In the present work, we report the outcomes of re-do metastasectomy in a large, contemporary, multicenter clinical trial.

2 Methods

We retrospectively reviewed the files of 621 consecutive patients who underwent pulmonary metastasectomy at the Johanniter Hospital Treuenbrietzen and Hannover Medical School between January 2010 and December 2014. The criterion for inclusion into the study was re-do metastasectomy. Exclusion criteria were metastasectomies done without curative intent and a loss of follow-ups after metastasectomy, which consisted of checkups with computer tomography (CT) lung scans every 3 months.

The following information was retrieved from patients' files: age at first metastasectomy, gender, primary tumor, nonsurgical oncologic therapies, number of metastases and the largest diameter of metastases, completeness of metastasectomy, lymph node dissection, lymph node metastases, and postoperative complications. Additional follow-up data were obtained from the referring oncologist. A calculation of overall survival (OS) included every alive patient at the time of inclusion. A disease-free interval was defined as the time between the resection of the primary tumor and the first lung metastasectomy. Re-metastasectomy was defined as a lung re-do surgery in patients who had undergone prior pulmonary metastasectomy on the ipsilateral side. In bilateral approaches or bilateral relapse, the side of the first surgery was chosen as a reference. The standard surgical procedure in both clinics was laser metastasectomy. Following anterolateral thoracotomy, bimanual palpation was carried out to detect all metastases. For laser resection, a Neodym:Yag laser Limax 120 using the 1318 nm wavelength (KLS Martin GmbH + Co. KG, Freiburg im Breisgau, Germany) was used. Conventional resections included wedge resections, segmentectomies, lobectomies, and pneumonectomies.

To identify differences between categorical variables, Pearson's c^2 test with Fisher's correction was used. Survival curves were created with the Kaplan Meier method, and survival rates were compared with a log-rank test. To evaluate factors influencing overall survival, the Cox proportional hazards model was applied after empirical variable selection. A p-value of <0.05 defined statistically significant differences.

3 Results

Out of the 621 patients who underwent initial pulmonary metastasectomy, 64 patients aged 62.6 ± 12.8 years (F/M – 28/64) underwent repeat metastasectomy and these patients were included in the analysis (Table 1). The median follow-up was 44 (6–88) months. During the follow-up, 21 patients died. All the patients underwent a second metastasectomy (64/64), later 55% underwent a third metastasectomy (35/64), 19% underwent a fourth metastasectomy (12/64), and 9% underwent a fifth metastasectomy (6/64), bringing a total to 181 of re-do metastasectomies.

The primary malignancies of metastases were colorectal carcinoma 19 (29.7%), renal cell carcinoma 15 (23.4%), sarcoma 9 (14.1%), urothelial carcinoma 4 (6.3%), head and neck cancers 4 (6.3%), and others 13 (20.3%). The median duration from diagnosis of primary malignancy to the first diagnosis of pulmonary metastases was 39.3 (0.0–178.4) months. Of these, 12 patients (18.8%) had a disease-free interval of less than 1 year compared to 10 patients (15.6%) with a disease-free interval of 1–2 years and 42 patients (65.6%) with more than 2 years. Baseline tumor data was regarding the different tumor origins analyzed according to the UICC score grades I–IV. Twenty (31.3%) patients received additional radiation therapy, 4 (6.3%) neoadjuvant, and 23 (35.9%) adjuvant chemotherapy. In 7 (10.9%) patients synchronous liver metastases, and other synchronous metastases were detected in another 8 patients (Table 1).

Two patients received presurgery and four patients received postsurgery chemotherapy for the metastases. Twenty-seven (42.0%) patients developed recurrent metastases after metastasectomy. The sites of metastases were liver (3.1%), brain (3.1%), bone (3.1%), and other localizations (14.1%). Forty-one (64.1%) patients developed pulmonary recurrence after the second surgery, and 35 (54.7%) patients had surgery for the third time. Out of the latter group, 17 (48.6%) patients developed a third pulmonary recurrence and 12 of them (34.3%) underwent a fourth operation. Out of these 12 patients, 6 developed a further recurrence and 3 underwent a fifth operation, with no more recurrences.

The mean number of metastatic nodules resected was 3.3 ± 3.6 and that of histologically confirmed metastases was 2.5 ± 3.0. A solitary metastasis was resected in 74 (40.9%) cases and more than 3 metastases in 46 (25.4%) cases. The median size of metastases was 10.6 ± 24.4 mm. Eighty-six resections were performed on the right side. Staged bilateral approaches were necessary in 79 cases. A complete lymph node dissection was performed in 50% of cases. Five percent of the 970 resected lymph nodes showed malignancy. Postoperative complications occurred in 21 (11.6%) cases. They were prolonged parenchymal fistula lasting for more than 4 days ($n = 12$), pneumothorax ($n = 2$), pneumonia ($n = 2$), respiratory distress ($n = 2$), and other rare complications like pleural effusion, chylothorax, and hemothorax ($n = 3$). The median duration of hospitalization was 9 (3–63) days.

The disease-free intervals were as follows: 19.8 ± 12.6 months after the first lung metastasectomy, 18.6 ± 16.4 months after the second metastasectomy, 19.3 ± 18.7 months after the third metastasectomy, 15.3 ± 14.1 months after the fourth metastasectomy, and 13.5 ± 13.6 months after the fifth metastasectomy.

The univariate Cox-regression analysis determined bilateral metastases, age, gender, resection margin, the number of metastases (>2), synchronous metastases, chemotherapy of the primary tumor, and preoperative liver metastases as significant factors concerning survival. The multivariate analysis determined that the number of metastases of more than two is a negative prognostic factor ($p = 0.013$). This was confirmed in

Table 1 Baseline characteristics of patients with repeat pulmonary metastasectomies

	n (%)
Total	64 (100)
Age (years)	Mean 62.6 ± 12.8
<50	7 (10.9)
50–65	23 (35.9)
>65	34 (53.1)
Gender	
Female	28 (43.8)
Male	36 (56.3)
Disease-free interval	Mean 54.6 ± 49.7
<1 year	12 (18.8)
1–2 years	10 (15.6)
>2 years	42 (65.6)
UICC stage (primary cancer)	41 (64.1)
I	6 (9.4)
II	8 (12.5)
III	16 (25.0)
IV	11 (17.2)
Additional therapy for primary cancer	
Radiotherapy	20 (31.3)
Neoadjuvant chemotherapy	4 (6.3)
Adjuvant chemotherapy	23 (35.9)
Synchronous liver metastases	7 (10.9)

UICC Union for International Cancer Control – cancer staging classification

the Kaplan-Meier curve showing a significantly better survival in the case of fewer than two metastases ($p = 0.0001$) (Fig. 1). The Cox-regression analysis also identified the presence of more than two metastases as a prognostic factor concerning the risk of recurrent lung metastases.

The median overall survival was 66.0 ± 3.8 months, the 1-year survival rate was 100%, the 3-year survival rate was 82.3%, and the 5-year survival rate was 63.3%. The median survival related to the number of resections was 62.2 months after two surgeries, 66.1 months after three surgeries, 73.8 after four surgeries, and 55.8 after five surgeries.

The 5-year overall survival rates were 63.3% after the first surgery, 50.9% after the second surgery, 74.4% after the third surgery, 83.3% after the fourth surgery, and 60.0% after the fifth surgery (Fig. 2). There were no significant differences in survival after the respective number of resections ($p = 0.647$, log-rank test).

4 Discussion

Lung metastasectomy represents a widely accepted therapy option for selected patients with lung metastases originating from a wide range of primary tumors. The concept of oligometastatic recurrence has been invoked to explain good results of local therapeutic approaches despite a metastasized tumor stage (Niibe and Hayakawa 2010; Hellman and Weichselbaum 1995). Like in most other works (Sponholz et al. 2017; Hachimaru et al. 2016; Mizuguchi et al. 2016), in this study, we show that multiple metastasectomies are worthy of performing regarding favorable long-term survival (Table 2). This is consistent with the concept of oligometastasis. If the biology of cancer does not change from the first metastasectomy, then subsequent metastasectomies should have similar efficacy. However, Yang et al. (2020) have described a significantly higher recurrence-

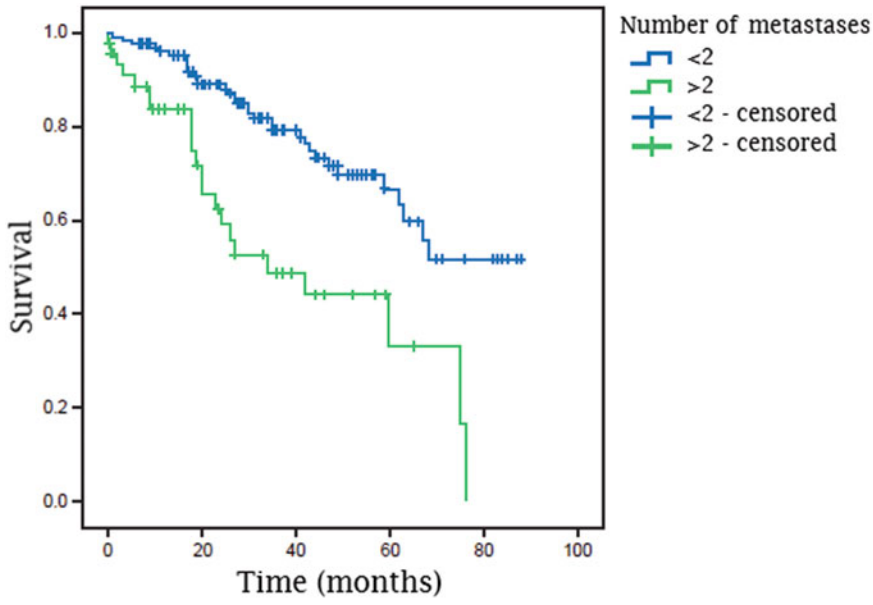

Fig. 1 Survival dependent on the number of metastases (≤2 vs. >2)

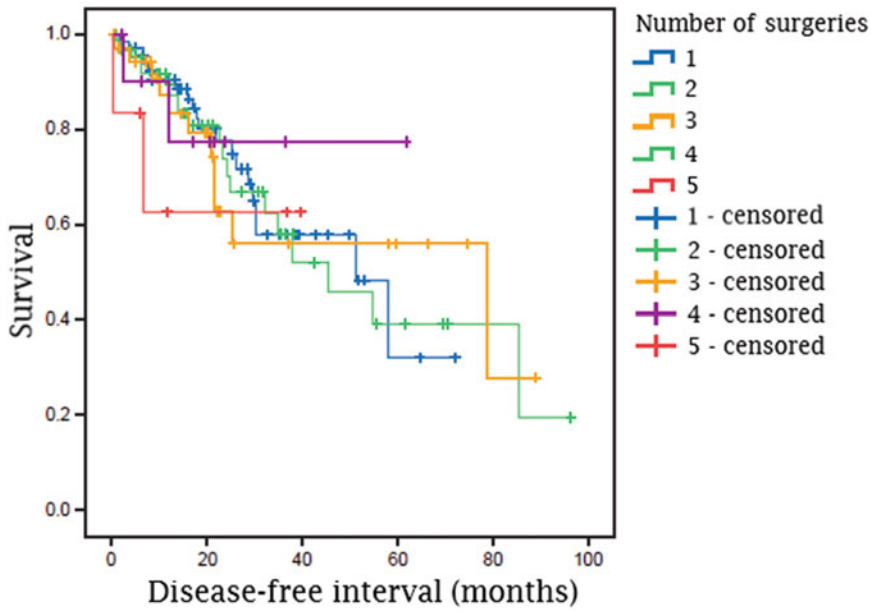

Fig. 2 Five-year overall survival rates depending on the number of metastasectomies

rate following the second re-metastasectomy as 13 out of the 14 patients died or had further recurrences within 12 months following the second re-metastasectomy. The authors conclude that the second metastasectomy is recommended as an optimal therapeutic option for the second recurrence, while further re-metastasectomies should be performed with caution as they may not enhance the survival time. Likewise, Mineo et al. (2015) have performed from two

Table 2 Selection of publications dealing with repeat pulmonary metastasectomies

Authors (year)	Primary tumor	Initial metastasectomy no. of patients/ 5-year survival	First recurrence	First re-do surgery no. of patients/5-year survival	Second re-do surgery no. of patients/5-year survival	Third re-do surgery no. of patients/5-year survival	Fourth re-do surgery no. of patients/5-year survival
Welter et al. (2007)	CRC		169/---	33/53.8%			
Park et al. (2010)	CRC	202/---		48/79.0%	10/78%		
Borasio et al. (2011)	CRC	137/55.4%		16/59.5%	5/---		
Salah et al. (2013) Meta-analysis	CRC	759/58%		148/57.9%			
Mineo et al. (2015)	Various	---/42%		113/65%	54/---	31/---	8/---
Hachimaru et al. (2016)	CRC	138/61.7%		33/64.3%			
Hishida et al. (2017) Multicenter[a]	CRC	898/---	216/898	132/75.3%	22/55.1%	2/---	
Mizuguchi et al. (2016)	HCC	19/48%	7/19	5/67% (3-year survival)			
Chudgar et al. (2017)	STS	539/---	341/539	141/---			
Sponholz et al. (2017)	CRC	238/48%	101/238	52/75%			
Cheung et al. (2018)	Sarcoma	42/20%[b]		20/37.4%[b]	12/63.6%		
Menna et al. (2018) Multicenter	CRC	203/34%		92/[c]	11/---		
Yamamoto et al. (2019)	STS/ osteosarc	44/43.5%		8/60%			
Yang et al. 2020[d]	CRC	248 [d]/74.8% (lung $n = 117$)	133 [d]/248 (lung $n = 58$)	52 [d]/62.8% (lung $n = 31$)	14 [d]/45.6% (lung $n = 9$)		
Matsumoto et al. (2019)[e]	CRC	1073/45% [f] (lung $n = 44$)	809/1073	323 all sites/43%	80 all sites/ 46%		
Present multicenter study	Various	621		64/50.9%	35/74.4%	12/83.3%	6/60.0%

CRC colorectal cancer, *HCC* hepatocellular carcinoma, *STS* soft tissue sarcoma

[a]Nationwide Japanese database

[b]5-year overall survival refers to the patient groups without further metastasectomy

[c]5-year overall survival following repeat metastasectomies showing no significant difference compared to patients with single metastasectomy

[d]Data referring to concomitant lung and liver metastases

[e]Data referring to the concomitant liver, lung, and peritoneal metastases

[f]Number of patients having all recurrences sites (liver, lung, or peritoneum) as opposed to the 5-year overall survival referring to patients with lung metastases only

metastasectomies in 113 patients, three in 51 patients, and so forth down to six re-do metastasectomies in three patients for lung metastases. They found that the more the resections, the greater the probability of recurrences and shorter the disease-free time, possibly making the re-do surgery lose its efficacy after the initial procedure.

A favorable effect on the long-term survival of repeat metastasectomies we found in this review is in line with several other studies. Cheung et al. (2018) have reported an increased median survival of 63.5 months after two and more repeat resections of pulmonary metastases in the sarcoma cohort of 243 patients. Park et al. (2010) have reported that out of 48 patients with colorectal cancer who underwent the second metastasectomy due to pulmonary recurrence, the overall 5-year survival was 79%, and in 10 patients who underwent the third metastasectomy it was 78%. According to Matsumoto et al. (2019), there is no difference concerning the overall 5-year survival following the first and second re-do procedures (43% vs. 46%), whereas Hishida et al. (2017) report a significantly enhanced survival following the first re-do surgery when compared with the second one (75.3% vs. 55.1%) (Table 2).

Presumably, patients with the favorable outcome have not overrun a specific biologic condition of the malignant disease. An interesting issue would be to identify this condition or a threshold over which metastasectomies are of no further benefit. The issue is confounded by differences in prognostic criteria following repeat metastasectomy from one publication to another. According to the present data, more than two metastases were associated with a higher risk of relapse. Other publications dealing with repeat metastasectomy confirm that the number of metastases is a strong negative predictor (Mineo et al. 2015; Salah et al. 2013; Borasio et al. 2011; Welter et al. 2007). Other studies have identified nodal status (Menna et al. 2018), the diameter of the largest metastasis (Yamamoto et al. 2019), concomitant liver metastases (Hishida et al. 2017), or disease-free survival (Yang et al. 2020; Mineo et al. 2015; Borasio et al. 2011) as

Table 3 Significant negative predictors for oncologic outcomes after repeat lung metastasectomies

Authors (year)	Negative predictor 1	Negative predictor 2	Positive predictor
Welter et al. (2007)	Number of metastases		
Borasio et al. (2011)	One vs multiple metastases	DFI <24 months	
Salah et al. (2013)	Number of metastases >3	>3 cm	
Mineo et al. (2015)	Multiple metastases	Short DFI	
Hachimaru et al. (2016)	High serum CEA level prior to re-metastasectomy		
Hishida et al. (2017)	Concomitant liver metastasis	Primary location in the rectum	
Chudgar et al. (2017)[a]	Preoperative chemotherapy prior to re-metastasectomy	R1/R2 metastasectomy	
Sponholz et al. (2017)	Low grading of primary tumor		
Menna et al. (2018)	Nodal status		Adjuvant chemotherapy
Yang et al. (2020)	DFI <6 months		
Yamamoto et al. (2019)	>2 cm (largest nodule)	DFI <12 months	
Present multicenter study	Number of metastases >2		

DFI disease-free interval, *CEA* carcinoembryonic antigen, *R1/R2* resection to microscopic residual tumor/macroscopic residual tumor

[a]Patients with soft tissue sarcoma, a negative predictor for the first re-metastasectomy. Incomplete resection is detected as a negative predictor and is not specifically mentioned in almost all publications

negative predictors as well (Table 3). These data as heterogeneous as are conclusions on metastasectomies in general.

In conclusion, this work supports the notion of a survival benefit of repeat lung metastasectomy. A close interdisciplinary co-operation is of paramount importance for the development of a therapy schedule and the implementation and surveillance of the oncologic treatment. A systematic oncologic follow-up aiming at early detection and consequent local therapy of recurrent metastases may improve survival in selected patients.

Conflicts of Interest The authors declare no conflicts of interest concerning this study.

Ethical Approval This chapter based on a retrospective review of medical files does not contain any direct studies with human participants performed by any of the authors. The work was approved by a local Ethics Committee at Hannover Medical School (IRB no. 2879-2015).

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Quality of Life of Patients After Laparoscopic Pyeloplasty Due to Ureteropelvic Junction Obstruction: A Long-Term Observation

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Abstract

This study aims to define the quality of life (QoL) of patients who had undergone laparoscopic pyeloplasty due to ureteropelvic junction obstruction. The QoL was investigated in 26 patients after pyeloplasty, on average, at a 7.5-year follow-up. The operation was performed in a single center between 2002 and 2009 and its effectiveness was confirmed by diuretic renography. The QoL was assessed using the World Health Organization Quality of Life (WHOQOL-BREF) questionnaire. Additionally, we used an own questionnaire, created for this study, specifically assessing the health-related quality of life after pyeloplasty. Overall, 96% of patients were satisfied with the surgical procedure and all would agree to have another pyeloplasty procedure if needed. In one case, dissatisfaction was caused by persisting postoperative pain. All patients but one, dissatisfied due to persisting pain, reported that the postoperative pain intensity was not a problem that would impact the QoL or professional activity. We conclude that laparoscopic pyeloplasty did not adversely affect the patients' QoL, which might stem from beneficial functional outcomes making the patients satisfied with treatment results.

Keywords

Laparoscopy · Pain · Pyeloplasty · Quality of life · Ureteropelvic obstruction

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1 Introduction

Minimally invasive surgical techniques gradually replace open surgery. Pyeloplasty is an example of progress. The first laparoscopic pyeloplasty was performed in 1993 (Kavoussi and Peters 1993; Schuessler et al. 1993), and since then the procedure has become the gold standard of treatment. Its clinical effectiveness has been confirmed in many a study (Strother and Mucksavage 2016; Autorino et al. 2014; García-Aparicio et al. 2014; Knoedler et al. 2013).

Currently, increasing attention is placed on the assessment of treatment effectiveness concerning the patient quality of life (QoL) in addition to the functional or survival results. There are often instances when the QoL declines despite improvements in laboratory indices or survival. The ureteropelvic junction obstruction is a common cause requiring laparoscopic pyeloplasty, surgical treatment of choice, but data on the postoperative QoL is scarce (Khoder et al. 2014; Parekh et al. 2008; Sahai et al. 2007). Therefore, in this study, we set out to define the health-related QoL in patients treated with laparoscopy for ureteropelvic junction obstruction at a long-term follow-up. We addressed the issue using the World Health Organization Quality of Life (WHOQOL-BREF) questionnaire, an abbreviated generic Quality of Life Scale, a tool that has not yet been used for this specific surgical entity, with the addition of an own health-related quality of life questionnaire, specifically addressing the after-pyeloplasty condition, created for this study.

2 Methods

Ninety-five patients who underwent transperitoneal unilateral laparoscopic pyeloplasty were originally invited to participate in the study. The time from surgery to the study follow-up visit was at least 5 years, with a mean of 89.8 ± 25.5 months. Twenty-seven patients responded to the invitation, but one of them having a horseshoe kidney was excluded from the study. The patient data were retrospectively obtained from hospital records. The elective surgery was performed in a single center by the same surgeon (TS) between 2002 and 2009, according to internationally recognized techniques (Panek et al. 2019; Krajewski et al. 2017; Szydełko et al. 2010, 2012). The Anderson-Hynes pyeloplasty was performed in 19 (73%), Y-V pyeloplasty in 6 (23%), and Fenger pyeloplasty in 1 patient (4%). The decision regarding the pyeloplasty method was made intraoperatively after visualization of the anatomy of the ureteropelvic junction area.

2.1 Description of Surgical Procedures

Twenty-three patients were symptomatic with hydronephrosis. Three patients with ureteropelvic junction obstruction and hydronephrosis were asymptomatic. Before surgery, patients had undergone preoperative examinations such as ultrasonography, diuretic renography, excretory intravenous urography (IVU), and computed tomography (CT) to confirm the diagnosis of ureteropelvic junction obstruction. The upper limit of the half-time tracer clearance was 12 min for nonobstructive and 20 min for obstructive systems in diuretic renography in which the diuretic furosemide (1 mg/kg/dose) was given after radionuclide administration at the times indicated above. The Foley catheter was removed on the second postoperative day. The closed suction drain was removed 1 day later providing that the drainage was <25 ml per day. The double-J stent was removed 4–6 weeks after pyeloplasty.

2.2 Follow-Up Visit Several Years After Pyeloplasty

At the scheduled follow-up visit, we evaluated each patient's medical history, such as the presence of arterial hypertension, metabolic diseases, or renal and diuresis complaints, and collected information on the passed perioperative period, with emphasis on postoperative complications, according to the classification by Dindo et al.

(2004). We asked patients to estimate the intensity of pain before surgery on a visual analog scale (VAS) of pain. The ruler-scale was 100 mm in length, with a score ranging from 0 mm (no pain) to 100 mm (extreme pain). The following cut-off distances were used: no pain (0–4 mm), mild pain (5–44 mm), moderate pain (45–74 mm), and severe pain (75–100 mm) (Hawker et al. 2011). We assessed the current pain intensity and performed an ultrasound examination of the affected kidney. Each patient was asked to fill out two questionnaires. The main questionnaire was the WHOQOL-BREF, version 1996, consisting of 26 questions. Two questions concern the general quality of life and health status. The remaining ones are divided into four domains: somatic, psychological, social, and environmental. All questions refer to the patient's feelings during the past 4 weeks. The numerical score of each domain reflects the perception of the quality of life represented by the domain. Two patients failed to complete the questionnaire. The other questionnaire was created for this study by one of the authors (WP) and consisted of five items specifically assessing the health-related quality of life after pyeloplasty. It consisted of five items:

1. Are you satisfied with the surgery outcome?
2. Would you agree to have another laparoscopic pyeloplasty if needed?
3. How do you estimate your current pain complaints on the VAS?
4. Does pain significantly affect your life and professional activity?
5. How many additional procedures did you undergo in the aftermath of laparoscopic pyeloplasty?

Besides completing the questionnaires, the patients underwent diuretic renography using the protocol akin to that used before pyeloplasty and the following blood tests: complete blood count, electrolytes, creatinine level, and the estimated glomerular filtration rate.

The pain associated with ureteropelvic junction obstruction before laparoscopic pyeloplasty was retrospectively rated and compared to the pain level on the VAS during the current follow-up visit. Continuous data were expressed as means±SD and differences between the mean values were evaluated using a *t*-test. Categorical data were expressed as counts and percentages. A p-value < 0.05 defined a statistically significant difference. The QoL was assessed after the laparoscopic pyeloplasty only.

3 Results

Out of the 26 patients participating in the study, 10 (38.5%) were men and 16 (61.5%) were women. The mean age at the time of surgery was 32.2 ± 10.6 years (range 16–56 years). In 12 (46.2%) patients, the crossing blood vessels were identified. They are aberrant vessels running to the lower pole of the kidney crossing the ipsilateral ureter most often anteriorly, which causes extrinsic compression of the ureter and obstruction of the renal collecting system. The patients' perioperative characteristics are presented in Table 1.

During the 6-week postoperative time, one significant complication of peritonitis was observed which was caused by a urine leak treated by subsequent laparotomy. Additionally, four patients required a change of a double-J stent. Other complications were infrequent and mild as detailed in Table 2.

In 23 out of the 26 study patients, the half-time tracer clearance was less than 12 min in diuretic renography performed during the study follow-up visit. Surgery resulted in a significant decrease in the perception of pain when voiding reported on the VAS, 6.5 ± 3.5 before to 1.4 ± 1.7 points after pyeloplasty ($p = 0.002$). Detailed follow-up outcomes are presented in Table 3.

Overall, 96% of patients were satisfied with the pyeloplasty procedure and would agree to repeat it if needed, despite that some suffered from postoperative complications. In one case, dissatisfaction was caused by an insufficient decrease in pain. All the patients reported that the low-grade pain persisting post-surgery did not significantly affect their life and professional activity. One patient underwent treatment of renal stones on the operated side a year after

Table 1 Perioperative characteristics concerning laparoscopic pyeloplasty

Sex; n (%)	Male	10 (38.5)
	Female	16 (61.5)
Surgery method; n (%)	Anderson-Hynes pyeloplasty	19 (73.0)
	Y-V pyeloplasty	6 (23.0)
	Fenger pyeloplasty	1 (4.0)
Kidney involved; n (%)	Left	14 (53.8)
	Right	12 (46.2)
Crossing vessels; n (%)	Present	12 (46.2)
	Absent	14 (53.8)
Surgery length; mean ± SD (min)	With crossing vessels	185.0 ± 36.1
	Without crossing vessels	203.2 ± 87.4
	Anderson-Hynes pyeloplasty	207.6 ± 70.3
	Y-V pyeloplasty	166.7 ± 51.3
	Fenger pyeloplasty	120.0
	Average duration of pyeloplasty	194.8 ± 68.0

Table 2 Postoperative complications after laparoscopic pyeloplasty in patients with ureteropelvic junction obstruction, according to the Clavien-Dindo classification of severity

Clavien-Dindo-grade	n (%)	Description	Treatment
I	5 (19.2%)	3 patients (11.5%) – pyrexia	Antipyretics
		2 patients (7.7%) – spontaneous prolapse of a double-J catheter	No treatment
II	1 (3.9)	Asymptomatic urinary infection	Antibiotics
IIIa	4 (15.4)	Obstruction of a double-J catheter with or without pyrexia	Catheter exchange
IIIb	0	–	–
IVa	0	–	–
IVb	1 (3.9)	Peritonitis due to urine leakage	Laparotomy
V	0	–	–

pyeloplasty (three sessions of extracorporeal shock wave lithotripsy) (Table 4).

The WHOQOL-BREF questionnaire demonstrated that patients evaluated the QoL as good (average 4 on 1–5 scale) and health condition as moderate (average 3.5 on 1–5 scale). Three (12.5%) patients were discontented with the health status (Table 5). Concerning the social domains in the WHOQOL-BREF questionnaire, the highest score was reported for the social relationship and lowest for physical health (Table 6).

4 Discussion

Minimally invasive pyeloplasty is the gold standard for the treatment of ureteropelvic junction obstruction. Clinical outcomes are excellent with the improvement observed in 85–100% of patients. Several surgical approaches appear equally effective (Janczak et al. 2018; Krajewski et al. 2017; Szydełko et al. 2012). The present study confirmed the good outcome of laparoscopic pyeloplasty as 88.5% of patients presented at the several-year long follow-up the correct urine passage from the renal pelvis into the ureter, which was confirmed by diuretic renography, and 80.8% of patients reported a reduction in painful urination.

Three of our patients did not have furosemide half-clearance time reduced to below 12 min. Each of them, despite not showing the expected outcome in diuretic renography, was satisfied with the pyeloplasty result, due likely to reduced

Table 3 Long-term follow-up outcomes after laparoscopic pyeloplasty in patients with ureteropelvic junction obstruction

Follow-up; mean ± SD (months)		89.8 ± 25.5
Age during control; mean ± SD, range (years)		39.3 ± 10.2 (21–64)
T½ - halftime to tracer clearance in diuretic renography; n (%), mean ± SD (min)	< 12 min	23 (88.5), 5.6 ± 2.3
	> 12 < 20 min	2 (7.7), 12.8 ± 1.0
	≥ 20 min	1 (3.9)
T½; depending on the presence of crossing vessels (CV); n (%), mean ± SD (min)	CV present	12 (46.2), 4.7 ± 4.1
	CV absent	14 (53.8), 6.0 ± 2.6
Hydronephrosis found in ultrasonography; n (%)	Absent	8 (29.6)
	Grade 1–2	11 (42.3)
	Grade 3–4	7 (26.9)
	Mean/median antero-posterior pelvis diameter (mm)	20.7/16.4
VAS (mean ± SD) (points)	Preoperative	6.4 ± 3.5
	Postoperative	1.4 ± 1.7
VAS reduction; n (%), mean ± SD (points)	Severe-to-mild	18 (69.2), (VAS from 7.6 ± 1.2 to 1.2 ± 1.2) p = 0.02
	Moderate-to-mild	3 (11.5), (VAS from 7.3 ± 0.6 to 3.0 ± 1.0) p = 0.04
	Severe-to-moderate	2 (7.7), (VAS from 8.5 ± 0.7 to 5.0 ± 1.4) p = 0.09
	Asymptomatic patients	3 (11.5%), (VAS from 0 to 0)

VAS visual analog scale of pain

Table 4 Results of own survey questionnaire for health-related quality of life

Are you satisfied with the pyeloplasty outcome?	Yes	25 (96.2%)
	No	1 (3.9%)
Would you agree to have another laparoscopic pyeloplasty if needed?	Yes	25 (96.2%)
	No	1 (3.9%)
How do you estimate your present intensity of the pain (points)	1.4 ± 1.7	
Does the pain significantly affect your life and professional activity?	Significantly	0
	Insignificantly	4 (15.4%)
	Not at all	22 (84.6%)
How many additional procedures did you undergo due to complications after pyeloplasty?	Antibiotic or antipyretic administration	3 patients
	Change of double-J catheter	4 patients
	Laparotomy	1 patient
	ESWL	1 patient

VAS visual analog scale of pain, *ESWL* extracorporeal shock wave lithotripsy

pain intensity when voiding. Nonetheless, they had to remain under close observation. Good clinical outcome, with the furosemide half-clearance time below 12 min and pain reduction, was also noticed in 18 patients with postoperative kidney hydronephrosis. Interestingly, a single patient who reported dissatisfaction with pyeloplasty due to an insufficient reduction in pain intensity did have the furosemide half-clearance time below 12 min and no kidney

Table 5 General subjective quality of life (QoL) and health status (HS) based on the World Health Organization Quality of Life (WHOQOL-BREF) questionnaire

Quality of Life			Health status	
Score	Patients (n)	%	Patients (n)	%
1	0	0	0	0
2	0	0	3	12.5%
3	4	16.7%	6	25.0%
4	16	66.7%	14	58.3%
5	4	16.7%	1	4.2%

Table 6 Domains of the World Health Organization Quality of Life (WHOQOL-BREF) questionnaire

	Score
Domain	Mean ± SD (range)
Physical health	54.1 ± 9.4 (38–69)
Psychological	68.0 ± 10.3 (44–88)
Social relationship	77.4 ± 9.4 (69–100)
Environment	65.8 ± 9.4 (44–81)

hydronephrosis. Likewise, most patients with other complications noted on the Clavien-Dindo grading scale reported a substantial pain reduction when voiding, satisfying QoL, and would agree to have another pyeloplasty procedure if needed.

The WHOQOL-BREF score in the health domain, somehow discrepantly, lagged the excellent surgical outcome of pyeloplasty. It reached an average of 3.5 points on the 1–5 scale, even though the global QoL score was better, reaching 4 points. A poorer perception of health could be influenced by comorbidities, which was not controlled for in the present study. Sahai et al. (2007) have shown a lack of difference between preoperative and postoperative physical QoL among patients who underwent laparoscopic upper urinary tract surgery for non-malignant conditions. However, there was a significant improvement in the postoperative mental QoL caused by relief from the emotional stress associated with pending surgery. Pace et al. (2003), comparing the effects of open and laparoscopic nephrectomy, have found that QoL one-year postoperatively returns to the baseline value faster in the laparoscopy-treated patients. In the present study, postoperative QoL was assessed at least 5 years after laparoscopy. Thus, it is a reasonable assumption that the patient's bias caused by perioperative stress and pain is negligible.

This study has several limitations. Data regarding preoperative and early postoperative periods were gathered retrospectively. The sample size was not big enough to perform a telling multifactorial analysis of factors affecting the QoL, concerning, for instance, the effects of surgical modifications or the presence of crossing vessels on the QoL. A low recruitment rate (26 out of the 95 patients invited to participate) could be caused by the fact that our clinic is one of a few reference centers in Poland performing laparoscopic pyeloplasty. Patients often come from distant locations, which limits their ability to participate. Additionally, a lack of symptoms in young adults, most patients were 30 years of age, makes them reluctant to undergo control tests. It seems unlikely that a low participation rate could result from poor surgery outcomes, considering a high success rate of laparoscopic pyeloplasty showed in this study. On the other hand, a success rate could introduce a "satisfaction" bias consisting of the greater willingness to participate in those patients who were satisfied when compared to dissatisfied, which could not be controlled for. Finally, the health-related QoL questionnaires were given to patients at the follow-up visit only.

Despite the limitations above outlined, we believe we have shown that patients suffering from ureteropelvic junction obstruction have a good clinical outcome associated with painful urination as well as psychological outcome concerning satisfaction with treatment results and quality of life at the long-term follow-up after laparoscopic pyeloplasty. The findings of this study may encourage both urological surgeons and patients in the decision-making for laparoscopic pyeloplasty to treat ureteropelvic junction obstruction.

Conflicts of Interest The authors of this manuscript have no conflicts of interest to disclose concerning this article.

Ethical Approval All procedures performed in studies involving human participants complied with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study was approved by the Bioethics Committee of Wroclaw Medical University in Wrocław, Poland; permit nr KB-458/2012.

Informed Consent All patients gave informed consent to participate in the study.

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Causes and Effects of Introducing Surgery Safety Checklist: A Review

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Abstract

The medical treatment process, particularly surgery, is inescapably bound to potential complications or undesirable adverse events. This narrative review aims to present the causes and effects of the introduction of the WHO Surgery Safety Checklist (SSC), the use of which is expected to reduce the number of perioperative errors, complications, and mortality. To achieve this objective, we performed a bibliometric analysis of medical citations indexed in the PubMed database using the SSC subject heading. Findings revealed a total of 1441 articles meeting inclusion status, with 1171 published during the last decade. After the screening of titles and abstracts, the members of the research team selected 75 articles, deemed most relevant for inclusion in the review, which were then thoroughly analyzed. All in all, the findings were that the use of SSC appreciably reduced the number of simple logistic errors in the perioperative period decreasing the frequency of resulting complications and mortality.

Keywords

Adverse effects · Mortality · Perioperative complications · Surgery safety checklist

1 Introduction

The treatment process, particularly surgery, is inescapably bound to potential errors and complications that may have a substantial bearing on outcome inclusive of fatality. Identifying the causes of such events, including death in the operating theater, is a difficult task. Most studies focus on risk factors resulting from the pathophysiological mechanisms of diseases. To substantiate the relationship to erroneous human activity is a challenge (Vincent et al. 2004). Nonetheless, difficult to explain undesirable events is most often attributed to medical staff (El Bardissi and Sundt 2012). To improve patient safety, the American Institute of Medicine and the National Academy of Engineering promoted the use of techniques tailored to assist the human factor. Tools were

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developed to improve and optimize the safety of medical activities (Boillat et al. 2019). Recent research shows that most often errors do not result from the actions of individuals but conflicting, incomplete, or suboptimal systems. The concept of the system is broad. It covers all logistic activities of people, technologies, equipment, materials, and the environment in which they occur (Carayon et al. 2006).

Multidisciplinary analyses indicate that the most common cause of adverse events in healthcare lies in the transmission of information that may be delivered incompletely, not at all, or to the wrong person. Further, problems existing for many years often remain unsolved until a crisis occurs (Zavalkoff et al. 2011). All these factors were considered by the WHO while developing guidelines for procedures necessary for patient safety, notably including patients subjected to surgery (WHO 2009). The guidelines took the form of the surgery safety checklist (SSC), a 19-item tool created in association with the Harvard School of Public Health in June 2008. This review aims to present the effects of the introduction of SSC on the number of perioperative complications and mortality. To achieve this objective, we performed a bibliometric analysis of medical citations indexed in the PubMed database as of November 2020 using the "surgery safety checklist" as the subject heading. We revealed a total of 1441 articles meeting inclusion status, 1327 of which were full-text publications. There were 1171 articles dated in the last decade. Among them, there were 54 clinical trials and 231 review articles. The members of the research team screened the titles and abstracts of 811 articles of the last decade and selected 75 of them, deemed most relevant, which were thoroughly analyzed for inclusion in the review.

2 Reasons for Introducing the Surgery Safety Checklist (SSC)

The WHO conducted large-scale research on the outcomes of surgical treatment. The preliminary results became known at the beginning of the twenty-first century. The findings are that perioperative mortality in patients undergoing major surgical procedures in industrialized countries is low (0.01%) (Braz et al. 2009), but the prevalence of significant undesirable events and complications reaches an alarming proportion of 3–16% (Weiser et al. 2008). These complications could have been preventable in one-half of the cases. For comparison, the most underdeveloped countries of Sub-Saharan Africa grapple with the mortality of 0.67% in patients during general anesthesia. African countries also lead in perioperative complications due to a shortage of licensed medical staff, equipment, and medicines, as well as the combination of simple organizational mistakes or unfavorable factors that should never happen. For instance, communication and teamwork issues are responsible for as many as 70% of undesirable events in obstetrics (Ogunlusi et al. 2017; Nwosu 2015; De Vries et al. 2008).

According to the estimations of independent authors as well as governmental agencies or WHO, 234 M surgical operations are performed annually in the world. Presuming a 10% perioperative rate of significant undesirable events, the issue comes down to the population exceeding one-half of the average European country, like Poland. Concerning fatalities, the number comes to about 1 M, globally, in the periprocedural or postoperative period (Weiser et al. 2008; WHO 2008a). The socioeconomic impact of this number of undesirable events is enormous. The important factor is, however, that a large proportion, reaching one-half of complications, could have been avoided. Numerous researchers, including WHO experts, indicate the possibility of a significant reduction in the number of errors made, using the simplest methods strengthening the hygienic and organizational milieu of operative theaters. An outstandingly high rate of perioperative complication and mortality in Sub-Saharan countries above outlined was one factor behind the introduction of the WHO SSC. A notorious shortage of qualified medical personnel compounds the issue (Collins et al. 2014; Vivekanantham et al. 2014). In the 2000s, WHO estimated the global shortage of health workers at

4.2 M, with 1 M new workers needed immediately in Sub-Saharan Africa alone (Crisp et al. 2008; Chen et al. 2004). Of the 64 selected hospitals in East Africa, only 4% had an adequately trained anesthesiologist, 92% had one anesthesia nurse at the most, and 8% had "anesthesia assistants" who substituted for trained anesthsiologists and were able to manage only a few selected procedures. The hospitals had to ask for help from neighboring ones. It was only in the last 10 years in Uganda, a country of about 40 M, that the number of anesthesiologists increased from 13 to 50, who work mainly in large urban areas (Hodges et al. 2007). Besides, the priority was to ensure safe anesthesia during delivery and C-sections, which is in line with WHO recommendations (Epiu et al. 2016). Concurrently, anesthesia standards have been worked out and published to be applied not only in tertiary hospitals but also in referral hospitals where medical care is provided for the largest chunk of the population (Epiu et al. 2017b). Similar studies and analyses were conducted in five national hospitals of the highest reference level in East Africa in Uganda, Kenya, Tanzania, Rwanda, and Burundi. Disappointingly, it has been found that none meets all the World Federation of Societies of Anaesthesiologists (WFSA) requirements for ensuring safe obstetric anesthesia (Epiu et al. 2017a). Anesthesia requires the presence of a doctor. The mere availability of the equipment is not enough. This is due to the need for comprehensive knowledge of the physiology and understanding of the anesthetic process itself. The doctor is necessary to ensure the safety of the entire anesthesia process, particularly the patency of airways, and to cope with hypo- or hyperventilation (Enright and Merry 2009). A 2016 review has reported that anesthesia contributes to 13.8% of all maternal deaths following C-sections. The mortality is almost twice as often when anesthesia is performed by non-anesthesiologists. Of all deaths, 76% results from complications in the respiratory tract, while the remaining cases are due to the employee's incompetence or logistic errors (Sobhy et al. 2016). Challenges faced by anesthesiologists and surgeons in many low-income countries include working in underinvested hospitals and shortages of staff, trained anesthesiologists and surgeons, equipment, materials, and often essential medications (Melekie and Getahun 2015; Grimes et al. 2011). A combination of unfavorable factors and the overwrought surgical and anesthetic teams lead to an increase in the prosaic medical errors (Collins et al. 2014; Vivekanantham et al. 2014). With the existing shortages and deficiencies, more complex cases must be treated in larger centers, which leads to excessive workload there and becomes another potential cause of adverse events (Barnes-Josiah et al. 1998).

In synopsis, doctors working in the countries of Sub-Saharan Africa face a significant shortage of equipment, particularly necessary for safe anesthesia. That applies to both surgery and childbirth. The problem is amplified by a shortage of staff necessary to ensure effective perioperative care. Additionally, shortages in these countries also concern trained and competent suppliers of equipment and service thereof. Therefore, key resources ensuring safe healthcare are missing. Reports indicate that only 5% of hospitals meet the requirements for operating and recovery rooms set by the World Federation of Societies of Anaesthesiologists (WFSA). Attention has also been directed to significant shortages of blood and blood products necessary for the safe conduct of procedures (Epiu et al. 2016). The issues outlined above have been the background for the introduction of the SSC.

Problem-solving efforts in the first place involve the implementation of the simplest solutions that do not require large financial outlays. It has been assumed that the introduction and obligatory use of a specific checklist would help put procedures on halt in case of shortages of staff and equipment, or ambiguities concerning the place and type of surgery. Due to the existing shortage of physicians in Africa, the focus was put on the training of non-physician staff who could take over the role of SSC coordinators and supervisors. Such a person should acquire the formal qualifications and skills of a group leader and be empowered to halt ongoing procedures, e.g., stop the surgery. Incidentally, women turned out to be the most suitable candidates for this role

from a psychophysiological standpoint. That, however, has required a turn-around in the hitherto stereotypical mental attitude of African people where men distinctly predominate in professional activities (WHO 2008b). Currently, many training programs improve patient safety and, simultaneously, develop leadership skills (Weaver et al. 2013). These activities vary by country and hospital, but some elements are common such as training by hospital board members and managers, or by healthcare risk leaders concerning the patient's safety (Schwendimann et al. 2013; Frankel et al. 2008; Thomas et al. 2005).

3 Principles of Surgery Safety Checklist (SSC)

The guiding principle of SSC is to help improve communication within the team and shape teamwork, reduce the number of perioperative errors and complications, and thus improve the patient's safety and treatment outcome. The WHO, introducing the SSC, outlined ten basic goals of safe surgery. Based on them, a 19-point questionnaire was created which systematizes verification procedures. Three stages of SSC use were distinguished. The first one refers to the time before the induction of anesthesia, the second before the first incision, and the third before the patient leaves the operating theater. Recommendations for the first stage are as follows: verification of the patient's identity, confirmation of consent to the surgery, confirmation of the place and type of the planned surgical procedure, confirmation of the airway assessment, and the inspection of equipment. The second stage consists of the confirmation of individual roles in the procedure, analysis of possible emergencies, surgery duration, and antibiotic prophylaxis. The final stage concerns the procedures related to the way the patient leaves the operating theater. During or immediately after wound closure, before the patient is taken away, the remaining surgical supplies and equipment must be checked to ensure nothing is left behind, results of any intraoperative examinations archived, and basic postoperative recommendations set forward. The introduction of SSC has been associated with a reduction in operational errors and lower rates of undesirable events worldwide, including low and middle-income countries (DeMaria and Neustein 2010; Seiden and Barach 2006; Gibbs 2005). The introduction and success of SSC are liable to stem from the psychological aptitude to follow imposed recommendations, making the staff act accordingly. For instance, as Epiu et al. (2016) have noted, over a century-old recommendation "wash your hands with soap" resulted in a dramatic drop in the perioperative infection rate. Contemporary estimates are that the implementation of SSC helped avoid eight deaths and save about $ 2 M in healthcare costs in just one hospital.

4 Knowledge and Effects of Surgery Safety Checklist (SSC)

As is the case with any tool, employees must be aware of its existence to use it. The WHO has put a lot of effort into spreading the knowledge about the existence of SSC (WHO 2008b). At present, the SSC is well known and widely used not only among specialists in the USA and EU but also in other countries of the world (Epiu et al. 2017a, b; Epiu et al. 2016; Sobhy et al. 2016; Melekie and Getahun 2015; Grimes et al. 2011; DeMaria and Neustein 2010). Even in a rather distant from the mainstream surgery field of dental implantology at the School of Dental Medicine of the University of Connecticut, the SSC has been familiarized with, implemented, and its usefulness in the prevention of perioperative threats presented in a special promotion program (Remiszewski and Bidra 2019). Surveys conducted in Africa and South and Central Americas show that SSC is well known as its existence has been confirmed by over 90% of respondents (Ogunlusi et al. 2017; Haynes et al. 2011).

According to the literature, the most common reaction to SSC among medical staff is a positive

attitude (93%) following the initial hesitation (Vivekanantham et al. 2014). It is believed that the SSC increases the interest in the issue of perioperative patient safety (Sucupira et al. 2016). Research conducted in Finland, which is a representative reflection of the whole of Scandinavia, shows that the implementation of SSC significantly increases the awareness of surgical teams in this regard, leading to the revisions of procedures in place and possible threats (Takala et al. 2011). Another approach, often encountered in underdeveloped countries, may be exemplified by Nigeria where about 93% of respondents know about the usefulness of SSC but are dissatisfied with the insufficient use of its recommendations in hospitals (Ogunlusi et al. 2017).

The authors dealing with the SSC issues focus on communication and interpersonal cooperation within the multidisciplinary surgical-anesthetic team. In principle, a team is a group of professionals united by one basic goal, which is the patient safety and optimal performance of the procedure. The overwhelming majority of respondents find that the SSC is conducive to intra-team communication (Santana et al. 2016; Russ et al. 2015; McDowell and McComb 2014; Berrisford et al. 2012; De Vries et al. 2011; Kearns et al. 2011; W-Dahl et al. 2011; Abdel-Galil 2010). An integrated team is gradually formed, engaged in joint work. This is achieved by training, which is particularly important for the originally disintegrated group of people (Melekie and Getahun 2015).

5 Implementation of Surgery Safety Checklist (SSC)

The way the hospital management implements the SSC procedures varies. At one extreme, there is the institutional compulsion and pointing to the obligations resulting from the employment contract, and at the other, the issue may be downplayed (Bosk et al. 2009; Hales and Pronovost 2006). At any rate, filling out the SSC documentation takes no more than 3–5 min, causing no distraction from the predefined duties (Russ et al. 2013). The implementation of SSC, like any other novelty, may cause some reluctance, particularly that new protocols are most often seen by medical staff as an increase in the number of a pile of existing duties. High routine uses of SSC, reaching 86%, takes place in university hospitals, which may result from a better organization of appropriate training and a more positive attitude of clinicians to new products (Vivekanantham et al. 2014). Non-university centers, particularly hospitals in the rural areas inhabited by low-income populations, face challenges such as an overload of patients and staff and equipment shortages, the circumstances that do not facilitate the use of SSC. However, SSC increases the probability of eliminating logistic errors in such conditions, so that it is essential healthcare professionals knowingly use it (Conley et al. 2011).

According to Ogunlusi et al. (2017), SSC is useful and lives up to its expectations in both developed and developing countries. A progressively increasing number of surgical procedures in developing countries have directed attention to strategies improving patient safety, particularly, when key resources like the availability of qualified medical staff are limited. The professional experience is still small there as only 68% of staff work in the hospital for less than 5 years. A survey performed among several dozens of surgeons, anesthesiologists, and operating theater nurses in Nigeria shows that 83% of them confirm knowledge of SSC. Among the respondents, however, only about 22% correctly states that the main purpose of SSC is to ensure patient safety by, inter alia, proper conduct of surgery. This shows that the reported over 80% knowledge of SSC is based on false assumptions as it is unlikely that the surveyed staff has comprehended the SSC sense. The main difficulties in the optimal use of SSC indicated by the respondents are the lack of training (58%), staff assertiveness (58%), and the inability to complete the paperwork in the required time frame (47%). These results show that more training and education on the use of SSC are necessary. A study performed in Ethiopia has shown that in about 40% of cases, SSC enters the

mandatory use after a short basic training which is not followed up (Melekie and Getahun 2015). Likewise, Sewell et al. (2011) have shown the lack of training lies at the heart of non-use or misuse of SSC in more than one-half of instances. Joy et al. (2011) have shown that training in SSC conducted in classrooms and simulator laboratories increases the use of recommended algorithms and contributes to better treatment outcomes. Nonetheless, surgical teams often resist participating in such activities. Changing the organizational culture of the operating theater team appears a challenging endeavor. The logistic issue that should be solved during training is the elimination or shortening of unnecessary time breaks and delays that affect the number of surgical treatments by improving non-technical skills in the operating theater. It is necessary to adjust these activities allocating them to specific teams, where some staff has been working and cooperating for many years and some appear and disappear after a short while. Other authors indicate a need for conducting regular audits of problems related to the use of SSC and providing constructive feedback on an ongoing basis, which should be based on coaching rather than typical regular checks. The emphasis is put on a stress-free continuous training (Hancorn and Blair 2010; Birkmeyer and Miller 2009; Haynes et al. 2009).

6 Surgery Safety Checklist (SSC) and Treatment Outcomes

The reports show surgical treatment outcomes and patient safety have improved after the introduction of SSC (Santana et al. 2016; Mayer et al. 2015; McDowell and McComb 2014; Kearns et al. 2011; Abdel-Galil 2010; DeMaria and Neustein 2010), with a clear decline in undesirable events. For instance, in Tunisia, a country with a modest income and work culture hardly compatible with the Anglo-Saxon standards, the eyebrow-raising 60% of undesirable events have been prevented by using the SSC as reported in a large retrospective study (Letaief et al. 2010). The most frightful undesirable event is death. A survey performed in the South Carolina hospitals has shown a reduction in the number of surgery-related deaths in the first 3 years after the implementation and completion of the SSC-based quality improvement program when compared with hospitals that do not use such algorithms. This also speaks for the increased patient safety while using the SSC (Haynes et al. 2017). The most informative studies show that the correct systematic use of SSC reduces the number of complications and mortality from 19.9% to 11.5% and 1.6% to 1.0%, respectively (Haugen et al. 2015; Haynes et al. 2009). The improvement in outcomes has been also shown in Scottish hospitals participating in the Patient Safety Program (SPSP) assessing the emergency surgeries performed in 2000-2014. The assessment concerned a cohort of 6,839,736 patients who were operated on, out of the 12,667,926 admitted to hospitals. A distinct overall decline in a surgical mortality rate of 36.6% was noted in surgical patients, remaining unchanged in non-surgical ones, since the implementation of SSC, with the annual mortality rate decreasing by 0.003% and 0.069% before versus after implementation, respectively (Ramsay et al. 2019).

A large study on the clinical role of SSC was also performed in a culturally different environment in China. The analysis covered 7209 patients with gastrointestinal cancer who underwent elective surgery at the Affiliated Hospital of Qingdao University. Data on patients who underwent surgery before the introduction of SSC were gathered retrospectively (3238 consecutive surgeries) and prospectively in those treated after its introduction (3971 surgeries). The incidence of complications, in-hospital mortality, and the median hospitalization duration before and after the SSC introduction, the latter verified by mandatory SSC documentation, were 16.4% versus 14.3%, 0.46% versus 0.18%, and 9 versus 8 days, respectively. Multivariate analysis shows the SSC was an independent factor decreasing the number of postoperative complications (odds ratio = 0.86; 95% confidence intervals 0.75-0.99) and thus, improving surgical outcome in gastrointestinal cancer (Wang et al. 2019). Likewise, other studies have also reported that the introduction of SSC or

other checklists is associated with a reduction in postoperative complications (De Vries et al. 2010; Vats et al. 2010; Edmondson et al. 2001). The use of SSC is particularly useful in "acute" cases and in situations requiring improvisation when there is the greatest probability of potential mistakes (Melekie and Getahun 2015). Additionally, the SSC helps avoid the confusion the patients may experience concerning the time and site of the planned surgery. A Guatemalan study has shown that the use of SSC decreases the chance of performing a mistaken procedure in the wrong patient (Ogunlusi et al. 2017; Ragusa et al. 2016). Unfortunately, despite more than 10 years of campaigning to prevent the happening of such erroneous events, they continue to crop up. Performing the wrong surgery or on the wrong body side still takes occasionally place.

Strangely enough, authors from France and the UK, where the SSC is mandatory, report the average number of correctly completed SSC documents is only 60% (Boillat et al. 2019; Fourcade et al. 2011; Warnock 2010). This finding seems a more complex and common issue concerning more countries than these two. Russ et al. (2013) have reported in their review that 50% of respondents do not fully follow the algorithms that are part of SSC, which means the documents are not completed or procedures required before the patient leaves the operating theater are not fulfilled. For instance, surveys conducted among Nigerian anesthesiologists, where the response rate was 83.6%, indicated the use of SSC by 62.7% of respondents, mainly in university hospitals (86.2% vs. 23.3% and 14.3% in other types of hospitals). Physicians who most often used SSC were aged 35-44 (approx. 71%) and the least often were over 55 years old (approx. 60%). Although the existence of SSC was known for 93.1% of respondents, a large proportion disbelieved that the algorithms may prevent errors and other undesirable events (Olatosi et al. 2018). Likewise, a Brazilian study has found that the use of SSC is widespread, reaching as much as 90.3% of surgeries, but almost in no case, the associated procedures would be fully done. Frighteningly, as many as 95.4% of surgeries were continued after errors in the implemented safety algorithms had been unraveled. The errors noticed concerned the timing of procedures to be performed and the continuation of activities despite the lack of key specialists. Further, the operating team did not actively implement the recommendations resulting from SSC which was treated just as another hassle to be completed (Almeida and Rodrigues 2019).

The negligence of safety practices increases the risks involved with surgery. This is supported by another Brazilian study involving 502 orthopedic physicians. The study found that about 41% of physicians had experienced an undesirable event such as performing surgery on the wrong patient or the wrong body side. As many as 25.6% of physicians indicated "misunderstanding" as the cause of a mishap. Despite that, as many as 35.5% of the surveyed physicians still failed to mark the body side for surgery before transporting the patient to the operating theater. All the research shows that such mistakes could be avoided by using SCC. While attempting to justify their misactions, physicians cited the lack of having savvy about all the features of SSC (65.3%) or training in this area (72.1%) (Motta Filho et al. 2013).

The implementation of SSC also appears of substantial help in counteracting surgery-related thromboembolic episodes. There are studies from various centers that confirm the effectiveness of SSC use in decreasing the clinical burden of these all too often happening and sometimes fatal episodes (Berrisford et al. 2012; W-Dahl et al. 2011). One other issue often discussed is the role of SSC in perioperative antibiotic prophylaxis. An important piece of work on the matter is a comparative analysis of the effects of using SSC during surgical procedures performed on the large intestine in two economically and culturally different countries; Canada and Brazil. The implementation of SSC algorithms was compared to the lack thereof considering the rate of surgical site infections. It decreased by a few percentage points in both countries. The authors emphasize the unequivocal benefit of implementing the SSC, with relatively low financial outlays (Gama et al. 2019). A reduction in the rate of perioperative

infections using the SSC standards has been also noticed in the investigation of other types of surgical procedures (Melekie and Getahun 2015; Boaz et al. 2014; De Vries et al. 2011; Haynes et al. 2011). Nonetheless, the success of antibiotic perioperative prophylaxis also depends, to a sizable extent, on specific hospital policies run by the specialized infection council or clinical bacteriologists, which usually differ by country and region. Antibiotic prophylaxis is adapted according to the microbial surveillance and the identification of pathogenic bacterial contaminants, and other local factors, all of which may go beyond and above simple compliance with the SSC algorithms. Therefore, antibiotic perioperative prophylaxis is a multifaceted process, the SSC is a major but unlikely a unique element of it (Siddiqi et al. 2019; Wæhle et al. 2019; Loozen et al. 2017; Vila et al. 2017).

In conclusion, the use of the WHO Surgery Safety Checklist, according to the overwhelming research, reduces the number of simple logistic errors in the perioperative period, thereby also error-related complications and mortality rate in surgical patients.

Conflict of Interest The authors declare no conflict of interest concerning this article.

Ethical Approval This review article does not contain any studies with human participants or animals performed by any of the authors.

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Benefit of Biological Drugs for Quality of Life in Patients with Ankylosing Spondylitis: A Systematic Review and Meta-Analysis of Clinical Trials

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Abstract

Ankylosing spondylitis (AS) is a chronic inflammatory rheumatic disease involving the axial skeleton, peripheral joints, and extra-articular manifestations like psoriasis, inflammatory bowel disease, or uveitis. A deterioration of quality of life (QoL) affects the disease management and therapeutic decision-making. This meta-analysis focused on the influence of biological drugs on the QoL in SA compared to the effects of other therapeutic modalities. We searched the databases of MedLine, Academic Search Ultimate, CINAHL Complete, and Health Source – Nursing/Academic Edition for articles related to AS treatment using the terms "ankylosing spondylitis" OR "rheumatoid spondylitis" OR "spondylitis" AND "quality of life" OR "patient-reported outcomes" OR "well-being" OR "health-related quality of life" OR "biological treatment". The search came up with 10 English-language articles published between 2010 and 2020. Patients were evaluated with the following indexes and questionnaires: Assessment of Spondyloarthritis International Society (ASAS), Bath Ankylosing Spondylitis Disease Activity Index (BASDAI), Ankylosing Spondylitis Quality of Life (ASQoL), 36-Item Short-Form Health Survey (SF-36), Functional Assessment of Chronic Illness Therapy-Fatigue (FACIT-F), and the Bath Ankylosing Spondylitis Functional Index (BASFI), and Bath Ankylosing Spondylitis Functional (BASFI) Indexes. We found that the QoL, assessed with the ASQoL, improved significantly better in patients treated with biological drugs when compared to those treated with other standard therapies or placebo at a 4-month follow-up. However, improvements in other disease characteristics could not be differentiated based on the therapy modality. The finding that biological drugs are superior in improving the QoL should strengthen the recommendations for their use in patients with AS.

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Keywords

Ankylosing spondylitis · Biologic drugs · Disability · Inflammation · Patient management · Quality of life · Rheumatic disease · Rheumatic disease · Therapy

1 Introduction

Ankylosing spondylitis (AS) is a chronic inflammatory disorder characterized by a broad spectrum of clinical manifestations, laboratory abnormalities, and imaging features. The prevalence of AS in the general population is between 0.1% and 6%. The disease affects young people, and its incidence is highest in people in their thirties. The AS is associated with the presence of the HLA-B27 antigen, found in 90–95% of patients (Zhu et al. 2019). The underlying mechanisms are related to inflammatory, infectious, immunological, and genetic disorders. Typical symptoms include pain in the spine, chest, and peripheral joints, fatigue, stiffness, physical disability, fever, weight loss, and shortness of breath. The symptoms are hardly specific, which leads to delayed diagnosis and treatment. The AS may also involve non-articular tissues, notably the heart, lungs, eyes, or digestive tract, increasing the risk of premature death (Reveille and Weisman 2013).

AS treatment is based on non-steroidal anti-inflammatory drugs (NSAIDs). When these drugs are ineffective and high disease activity persists, clinically demonstrated by the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) score > 4 and the elevated erythrocyte sedimentation rate (ESR) and plasma C-reactive protein (CRP), biological drugs can be used. They offer considerable hope for effective treatment. Particularly, tumor necrosis factor-alpha (TNF-α) inhibitors appear effective. The introduction of biologicals in AS treatment has enabled the effective containment of the inflammatory process in patients with highly active and aggressive disease (Gao et al. 2012).

Rheumatic diseases are associated with an increased risk of organ pathology but also with a progressive disability and increased psychological burden. AS symptoms affect all aspects of patients' daily life. This leads to disease progression and disability which compromises fitness to work, work quality, daily activities, and, generally, health-related quality of life (QoL).

The interest in QoL dates to the 1970s and is associated with the holistic view of medicine. Therapeutic interventions are expected to prolong life and improve its quality. According to the positive concept of health, it is not the mere absence of disease but good physical and mental functioning and social adaptation. In clinical practice, QoL assessment complements objective indicators of outcome. It facilitates the selection of an optimal treatment protocol from the existing options. QoL assessment is based on patient self-reporting. It shows limitations in functioning, identifies areas where the patient experiences such limitations, and suggests priorities and preferences that may be relevant to further management planning (Megari 2013). In rheumatology, QoL has significant clinical implications but is not often addressed in practice. Studies are often limited to the assessment of correlations between basic symptoms of pain and stiffness and QoL. Concerning the impact of AS on QoL, studies show that patients suffer from impaired physical and mental health (Yang et al. 2016). However, few studies have yet addressed the influence of treatment with biological drugs on AS symptoms and QoL.

Rheumatologic patients usually associate the notion of health with functional status and quality of living with the disease. Management planning follows the alternating sequence of disease remission and exacerbation. The characteristics of rheumatic diseases make it difficult to establish long-term treatment objectives. When seeking the optimal treatment protocol, besides ways to delay the development of joint lesions and alleviate the associated symptoms, one must seek to improve the patient's daily functioning and his perceived QoL. To this end, QoL assessment should be an essential part of patient management and therapeutic decision-making. The purpose of this meta-analysis was to assess the QoL of patients with

AS, with the particular emphasis on the influence of using biological therapy on QoL as compared to the effects of other therapeutic modalities.

2 Methods

2.1 Search Strategies

This meta-analysis concerned the English-language articles published between 2010 and 2020 which addressed the features and therapy of AS. We performed a systematic searched of electronic databases such as PubMed, MedLine, Academic Search Ultimate, AHFS Consumer Medication Information, Open Dissertations, CINAHL Complete Dentistry and Oral Sciences, GreenFILE, Health Source – Consumer Edition, Health Source: Nursing/Academic Edition, International Pharmaceutical Abstracts, and MasterFILE Premier using the terms "ankylosing spondylitis" OR "rheumatoid spondylitis" OR "spondylitis" AND "quality of life" OR "patient-reported outcomes" OR "well-being" OR "health-related quality of life" OR "biological treatment". The search followed the Cochrane guidelines and was consistent with the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) statement. Inclusion criteria consisted of age $\geq$18 years (7 studies), meeting the modified New York standards for ankylosing spondylitis (4 studies) or standards for axial ankylosing spondylitis in the Assessment of Spondyloarthritis International Society (ASAS) (3 studies), the need for daily treatment with NSAIDs or NSAIDs intolerance (4 studies), score $\geq$4 in the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI), pain score $\geq$4 on the numeric rating scale (NRS 0–10) (6 studies), and written informed consent (4 studies). Out of the several hundred articles identified, the search came up with 10 relevant studies, conducted in 15 countries on 5 continents. A detailed methodological flow diagram is presented in Fig. 1. Studies that contained incomplete data, case reports, reviews, lack of QoL assessment, and studies on children were excluded.

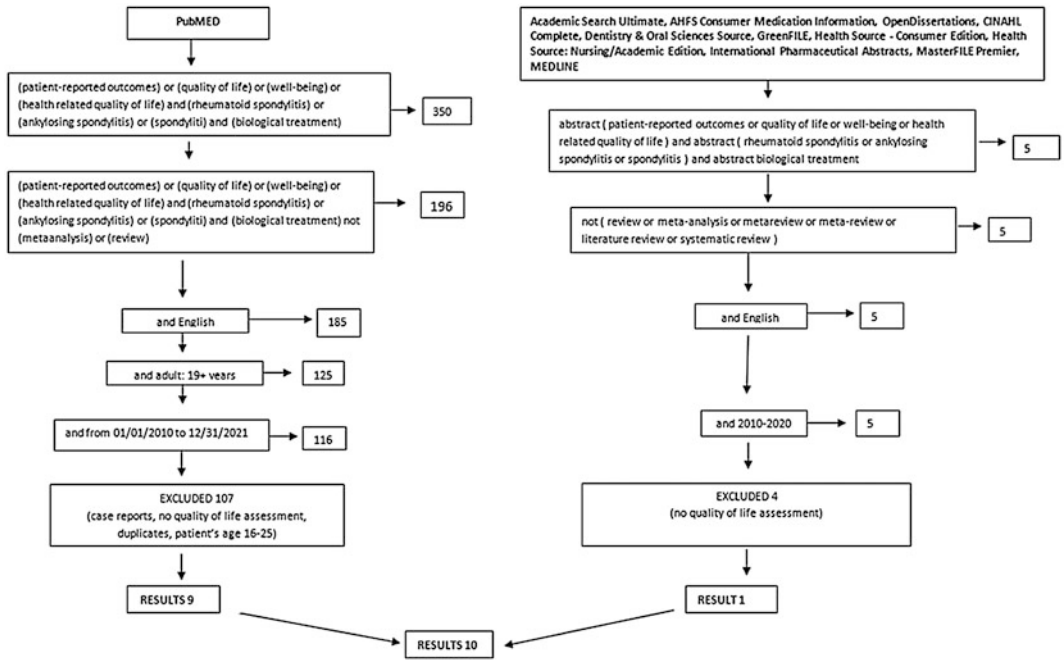

Fig. 1 Diagram showing the search-flow for the literature relevant for ankylosing spondylitis treatment

Questionnaire Structures The questionnaires used in the relevant studies were as follows: Bath Ankylosing Spondylitis Disease Activity Index (BASDAI), Ankylosing Spondylitis Quality of Life (ASQoL), 36-Item Short-Form Health Survey (SF-36), Functional Assessment of Chronic Illness Therapy-Fatigue (FACIT-F), and the Bath Ankylosing Spondylitis Functional Index (BASFI) (Rohde et al. 2020; van der Heijde et al. 2019; Fattahi et al. 2018a, b; Jafarnezhad-Ansariha et al. 2018; van der Heijde et al. 2018a, b; Arısoy et al. 2013; Pathan et al. 2013; Ertenli et al. 2012).

The ASQoL questionnaire is a disease-specific patient-reported outcome measure, based on the needs-model of QoL. It comprises 18 items, each with a dichotomous yes/no response option scored 1 and 0, respectively. A total score ranges from 0 to 18, with higher scores indicating a poorer QoL (Doward et al. 2003). The SF-36 is a 36-item questionnaire assessing the patient's health status. The questionnaire comprises eight domains: vitality, physical functioning, bodily pain, general health perception, physical role functioning, emotional role functioning, social role functioning, and mental health. Physical component score (PCS) and mental component score (MCS) may be calculated separately or along with eight domains of SF-36 Responses in each domain are converted to a scale of 0 to 100, with all items having assigned the same weight. The higher the score the better is the QoL (Ware Jr and Sherbourne 1992). The FACIT-F questionnaire covers four life domains of chronically ill patients. It comprises 5 parts: physical well-being (PWB) – 7 questions; social/family well-being (SWB) – 7 questions; emotional well-being (EWB) – 6 questions; functional well-being (FWB) – 7 questions, and fatigue subscale (FS) – 13 questions. Each question is rated on a five-point Likert scale. The higher the score the better is the QoL (Cella 1997). The BASFI questionnaire assesses the degree of functional limitation, with higher scores indicating a worse condition.

Literature articles were thoroughly screened by two reviewers for consistency with the subject of this meta-analysis above outlined. They determined if the content consists of interpretable patterns that sufficiently contribute to the accumulated knowledge and evidence-based practice and could advance the process of rehabilitation and care for SA patients. Any discrepancies in the reviewers' assessments were resolved by consensus. Data were extracted and put in the standardized forms that included general information, patient characteristics, study design, risk of bias according to the Newcastle-Ottawa Scale, and intermediate-to-long term (>6 months) main outcomes.

2.2 Study Groups

The active arm of this analysis included 593 patients (85.6% male) aged 22–60, suffering from AS for 1–21 years and treated with biological drugs. The main identified biological drugs used were the following: filgotinib (58 cases), adalimumab (92 cases), ixekizumab (164 cases), β-D-mannuronic acid (60 cases), apremilast (17 cases), infliximab (23 cases), and upadacitinib (89 cases). In the remaining cases, the generic nature of a biological drug was not provided.

The control group treated with other drugs included 702 (70.4% male) patients of 22–58 years of age, who suffered from AS for 1–18 years. They were treated by variable combinations of traditional drugs like NASIDs (582 cases), disease-modifying antirheumatic drugs (561) that notably included methotrexate (284 cases), analgesics (93 cases), and steroids (80 cases). In some studies, biological drugs were mixed with non-biological ones in an unstratified manner and were thus considered together. Aside from the used medications, 191 patients in the control group also received a placebo; 152 patients received a placebo in addition to other standard treatment and 39 patients received placebo alone.

2.3 Statistical Analysis

In this meta-analysis, we examined differences in the clinical improvements between SA patients treated with biological drugs (intervention group) and traditional non-biological drugs or placebo. In some studies, the drugs used were not stratified into types and thus were considered together. The effect-size (ES) was based on the standardized mean difference in a random or fixed model, based on the Q test of homogeneity. The random-effects model was used assuming that different studies would reflect different ES due to differences in patient samples and methods. In this model, the DerSimonian and Laird estimate with 95% confidence intervals (95%CI) were used, estimating the difference between the two groups based on the number of pooled standard deviations by which the two groups differed. This part of the analysis was performed using the R-software v3.6.2 for statistical computing for Windows. For the fixed model, Hedges' *g*, a bias-free measure of standardized mean differences, was used to estimate the ES, according to the formula: *g* x (mean-1 - mean-2)/SD*; where SD* is the pooled and weighted standard deviation of both study and control groups. The results were presented as forest plots. The total was calculated by assigning relative weights to treatment effects from the evaluated publications, depending on the sample size and standard error. A p-value of <0.05 defined a statistically significant difference. Calculations were performed using a commercial package of Statistica v13.3 software (StatSoft Inc; Tulsa, OK).

3 Results

3.1 Treatment of Ankylosing Spondylitis (AS)

The ASQoL assessment showed that patients treated with biological drugs showed a significantly better QoL at 4-month follow-up when compared to those treated with classical therapy alone (Hedges' *g* analysis for heterogeneity: df = 7, Q = 124.5, I^2 = 94.4%, p < 0.001, *vs.* df = 7, Q = 3.3, I^2 = 0.0%, p = 0.854, respectively), while both groups started from a similar baseline level of QoL (Fig. 2).

Since we found a beneficial influence on QoL of biological drugs in SA patients, we posed a question of whether the self-perceived improvement could relate to a particular biological drug used. The lack of standardization in studies on the effects of biological therapy makes it hard to compare patient outcomes. There are variably incomplete data, and different follow-up periods and instruments assessing the QoL and functional performance in the relevant studies surveyed. Nonetheless, we tallied the following information about the effects of single biological drugs.

Filgotinib – selective Janus kinase 1 (JAK1) inhibitor (*n = 58*) (van der Heijde et al. 2018a) – treatment follow-up at 3 months. ASQoL score: baseline 12.8 ±3.5, follow-up 8.0 ±5.2 – improvement by 4.8 points; SF-36 PCS (physical): baseline 33.1 ±5.6, follow-up 41.6 ±7.9 – improvement by 8.5 points; SF-36 MCS (mental): baseline 43.7 ±11.1; follow-up 47.7 ±9.3 – improvement by 4.0 points.

Ixekizumab – monoclonal antibody against interleukin-17A, (van der Heijde et al. 2018b) – treatment follow-up at 4 months. Q2W (*n = 83*): SF-36 PCS (physical): baseline 34.1 ±7.6, follow-up 42.1 ±0.8 – improvement by 8.0 points; Q4W (*n = 81*): SF-36 MCS (mental): baseline 34.0 ±8.0, follow-up 41.7 ± 0.8 – improvement by 7.7 points.

β-D-mannuronic acid – marine algal polysaccharide (*n = 30*) (Fattahi et al. 2018a, b; Jafarnezhad-Ansariha et al. 2018) **–** treatment follow-up at 3 months. ASQoL score: baseline 9.8 ±4.5; follow-up 6.6 ±4.6 – improvement by 3.2 points; baseline 9.1 ±0.7; follow-up 6.0 ±0.6 - improvement by 3.1 points; and baseline 9.8 ±4.5; follow-up 6.7 ±0.6 – improvement by 3.1 points, respectively.

Apremilast – phosphodiesterase 4 (PDE4) inhibitor (*n = 17*) (Pathan et al. 2013) – treatment follow-up at 3 months. FACIT-F score:

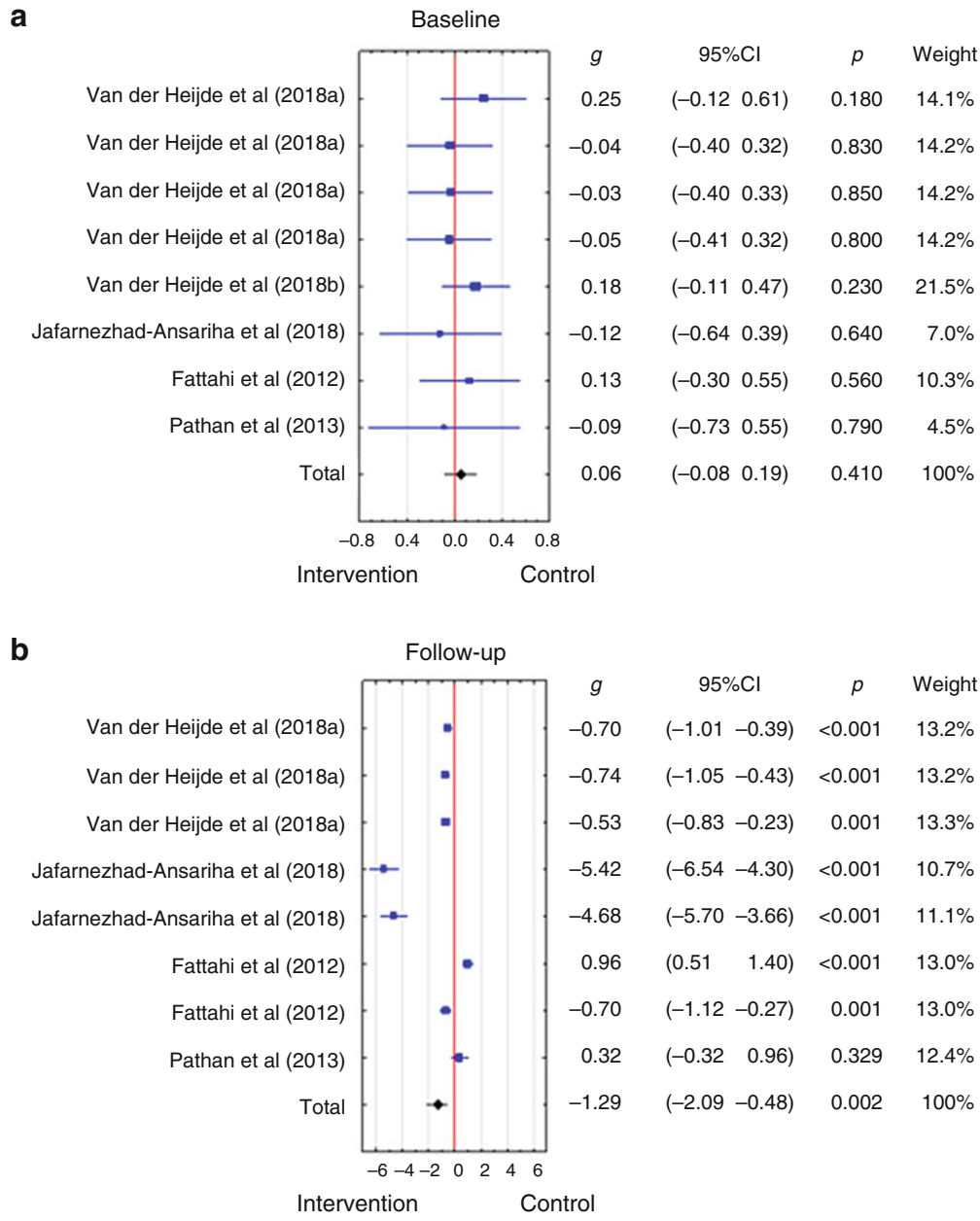

Fig. 2 Quality of life (QoL) in ankylosing spondylitis (AS) treated with biological drugs (intervention group) when compared to the control group not using biological drugs, assessed with ASQoL, except for a study of Pathan et al. (2013), where the FACIT-F questionnaire was used. Note no difference in QoL between the two groups at onset (**a**) and a distinctly better QoL at a 4-month follow-up (**b**)

baseline 107.8 ±25.7, follow-up 117.1 – improvement by 9.3 points. The improvement concerned the physical, social, and emotional domains, as well as the fatigue.

Upadacitinib – Janus kinase (JAK) inhibitor (Van der Heijde et al. 2019) – QoL improvement was verified based on the difference between the biological *versus* non-biological

treatment due to the unspecified treatment follow-up period.

The superior quality of life, assessed with the ASQoL questionnaire, was the single parameter found in this meta-analysis with benefits related to the implementation of treatment with biological drugs. All the other measurements showed improvements in response to treatment, irrespective of biological or traditional drugs used, or even placebo. Therefore, in further analysis, all the drugs were grouped. The AS disease activity, assessed on the BADSI scale, improved from baseline to follow-up while using biologicals (Fig. 3). However, a positive trend of improving the functional performance was also observed with traditional treatments as well as placebo, regardless of the treatment duration (Table 1).

Besides disease activity level, C-reactive protein (CRP) in blood plasma, a marker of inflammation in AS patients, is relevant for the monitoring of treatment effects. Figure 4 shows a significant drop in CRP levels after treatment with biological drugs ($p = 0.001$ for the entire group; Hedges' g analysis). A decline in CRP was noted while using all individual biological drugs included in the analysis except for one case when its level tended to rise after treatment with β-D-mannuronic acid (Jafarnezhad-Ansariha et al. 2018). However, CRP also declined while using other drugs, with a single exception of placebo in a study by Van der Heijde et al. (2018a). Detailed results for biological and other drugs are displayed in Table 2.

In the management of AS patients, the American College of Rheumatology (ACR) recommends the comprehensive ASAS assessment of the

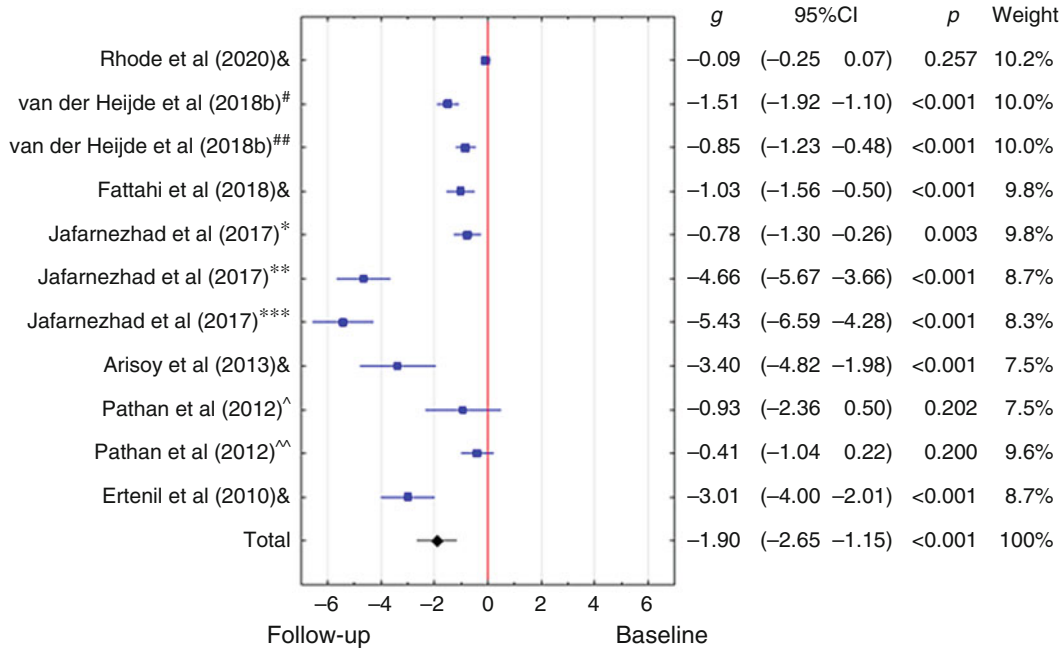

Fig. 3 Disease activity level in ankylosing spondylitis (AS) in patients treated with both biological and traditional drugs at baseline and follow-up after treatment, assessed with the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI). The random-effects model with Hedges' g. The effect size and confidence interval for each study appear graphically on a separate row (blue). The summary effect and its confidence interval are displayed at the bottom (black). 95% CI, lower and upper 95% confidence intervals; &drugs unstratified, #filgotinib, ##placebo, *β-D-mannuronic acid, **naproxen, ***placebo, ^apremilast, ^^placebo

Table 1 Disease activity level in ankylosing spondylitis (AS) in patients treated with both biological and traditional drugs at baseline and follow-up after treatment, assessed on the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) at baseline and follow-up after treatment, considering all drugs

Source	Treatment	Baseline		Follow-up		
		Patients (n)	Score	Months	Patients (n)	Score
Rohde et al. (2020)	Drugs unstratified	380	3.1 ±2.1	60	240	2.9 ±2.2
Van der Heijde et al. (2018a)	Filgotinib	58	6.9 ±1.2	3	58	4.5 ±2.0
	Placebo	58	7.0 ±1.3	3	58	5.6 ±1.9
Fattahi et al. (2018a)	Drugs unstratified	30	5.8 ±1.3	4	30	4.1 ±1.9
Jafarnezhad-Ansariha et al. (2018)	β-D-mannuronic acid	30	5.8 ±0.2	4	30	5.5 ±0.5
	Naproxen	28	5.7 ±0.2	4	28	3.9 ±0.5
	Placebo	27	5.9 ±0.2	4	27	3.8 ±0.5
Arısoy et al. (2013)	Drugs unstratified	9	7.0 ±1.1	4	9	2.1 ±1.6
Pathan et al. (2013)	Apremilast	17	4.8 ±2.2	4	17	3.2 ±1.6
	Placebo	19	4.4 ±1.8	4	19	3.6 ±2.0
Ertenli et al. (2012)	Drugs unstratified	16	6.0 ±1.2	4	16	2.3 ±1.2

Scores are means ±SD

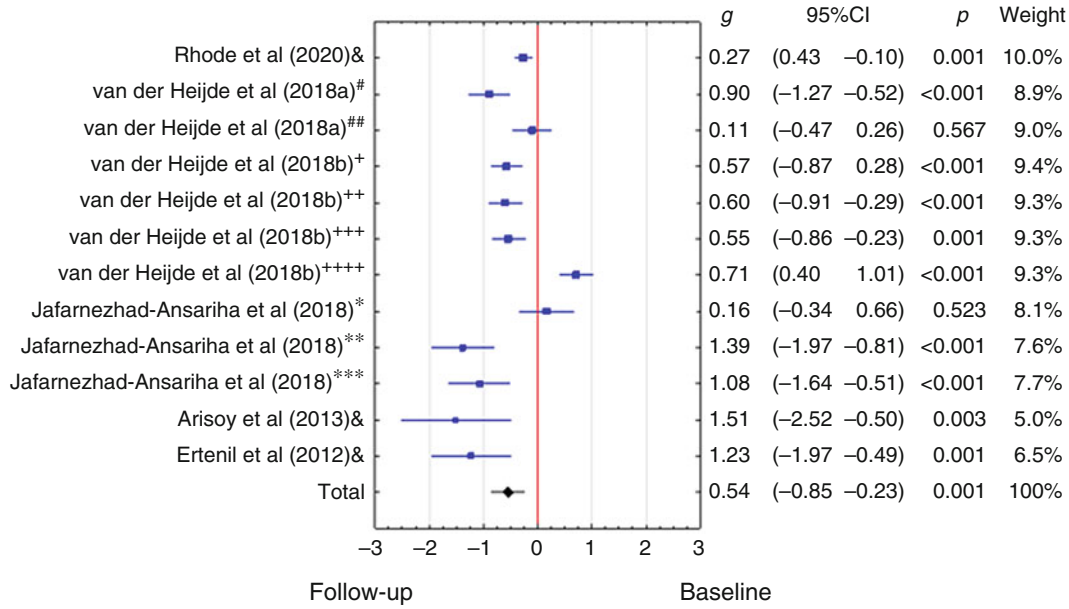

Fig. 4 C-reactive protein (CRP) in the blood plasma of ankylosing spondylitis (AS) patients treated with biological and traditional drugs at baseline and follow-up after treatment. The random-effects model with Hedges' *g*. The effect size and confidence interval for each study appear graphically on a separate row (blue). The summary effect and its confidence interval are displayed at the bottom (black). 95% CI, lower and upper 95% confidence intervals; &drugs unstratified, #filgotinib, ##placebo, +adalimumab, ++ixekizumab Q2W, +++ixekizumab Q4W, ++++placebo, *β-D-mannuronic acid, **naproxen, ***placebo

Table 2 C-reactive protein (CRP) in the blood plasma of ankylosing spondylitis (AS) patients at baseline and treatment follow-up, considering all drugs

Source	Treatment	Baseline		Follow-up		
		Patients (n)	mg/L	Months	Patients (n)	mg/L
Rohde et al. (2020)	Drugs unstratified	380	10.0 ±13.2	60	240	6.7 ±10.7
Van der Heijde et al. (2018b)	Filgotinib	58	19.6 ±13.3	3	58	8.8 ±10.5
	Placebo	58	21.2 ±23.0	3	58	18.9 ±20.2
Van der Heijde et al. (2018a)	Adalimumab	90	12.5 ±17.6	4	90	5.3 ±1.9
	Ixekizumab Q2W	83	13.4 ±15.3	4	83	6.8 ±2.0
	Ixekizumab Q4W	81	12.2 ±13.3	4	81	7.1 ±2.0
	Placebo	87	16.0 ±2.1	4	87	17.4 ±1.9
Jafarnezhad-Ansariha et al. (2018)	β-D-mannuronic acid	30	8.3 ±1.2	4	30	8.5 ±1.1
	Naproxen	28	8.0 ±0.8	4	28	6.8 ±0.9
	Placebo	27	8.1 ±0.9	4	27	7.0 ±1.1
Arzsoy et al. (2013)	Drugs unstratified	9	22.9 ±15.0	1.5	9	5.2 ±4.9
Ertenli et al. (2012)	Drugs unstratified	16	3.7 ±3.5	1.5	16	0.6 ±0.5

Scores are means ±SD

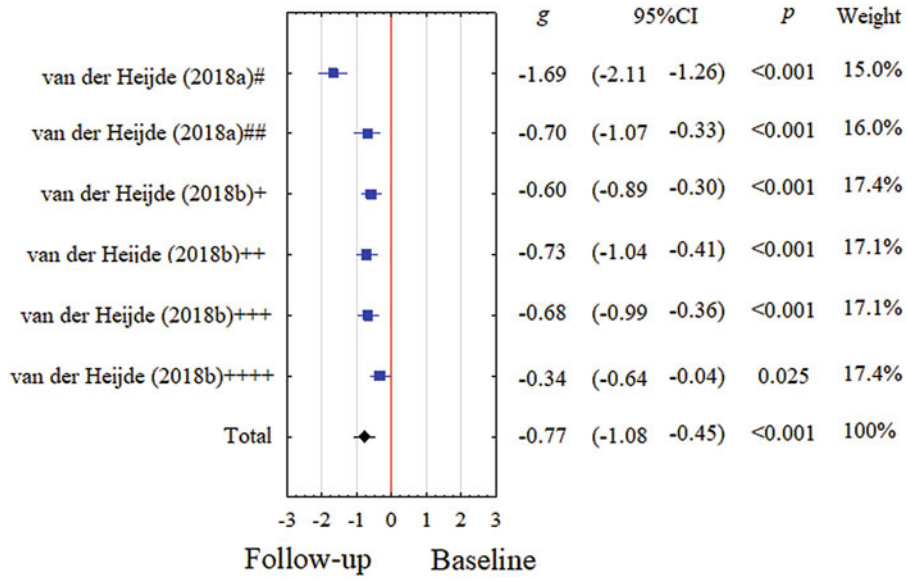

Fig. 5 Effectiveness of clinical treatment using biological drugs and placebo in ankylosing spondylitis (AS) patients, based on the SpondyloArthritis International Society (ASAS) assessment at baseline and follow-up after treatment. The random-effects model with Hedges' *g*. The effect size and confidence interval for each study appear graphically on a separate row (blue). The summary effect and its confidence interval are displayed at the bottom (black). 95% CI, lower and upper 95% confidence intervals; #filgotinib, ##placebo, +adalimumab, ++ixekizumab Q2W, +++ixekizumab Q4W, ++++placebo

patient's functioning, which is used as a criterion for initiating biological therapy (Ward et al. 2016). In our meta-analysis, a comparison of ASAS scores at baseline and follow-up after treatment showed a significant improvement in functioning in patients using biological drugs as well as in those receiving a placebo, with inappreciable differences (Fig. 5 and Table 3).

Table 3 SpondyloArthritis International Society (ASAS) scoring in ankylosing spondylitis (AS) patients at baseline and treatment follow-up, considering biological drugs and placebo

Source	Treatment	Baseline		Follow-up		
		Patients (n)	Score	(Months)	Patients (n)	Score
Van der Heijde et al. (2018b)	Filgotinib	58	4.2 ±0.6	3	58	2.8 ±1.0
	Placebo	58	4.2 ±0.8	3	58	3.6 ±0.9
Van der Heijde et al. (2018a)	Adalimumab	90	8.2 ±3.7	4	90	5.9 ±4.0
	Ixekizumab Q2W	83	8.4 ±3.6	4	83	5.7 ±3.9
	Ixekizumab Q4W	81	7.5 ±3.3	4	81	5.1 ±3.6
	Placebo	87	8.1 ±3.5	4	87	6.9 ±3.8

Scores are means ±SD

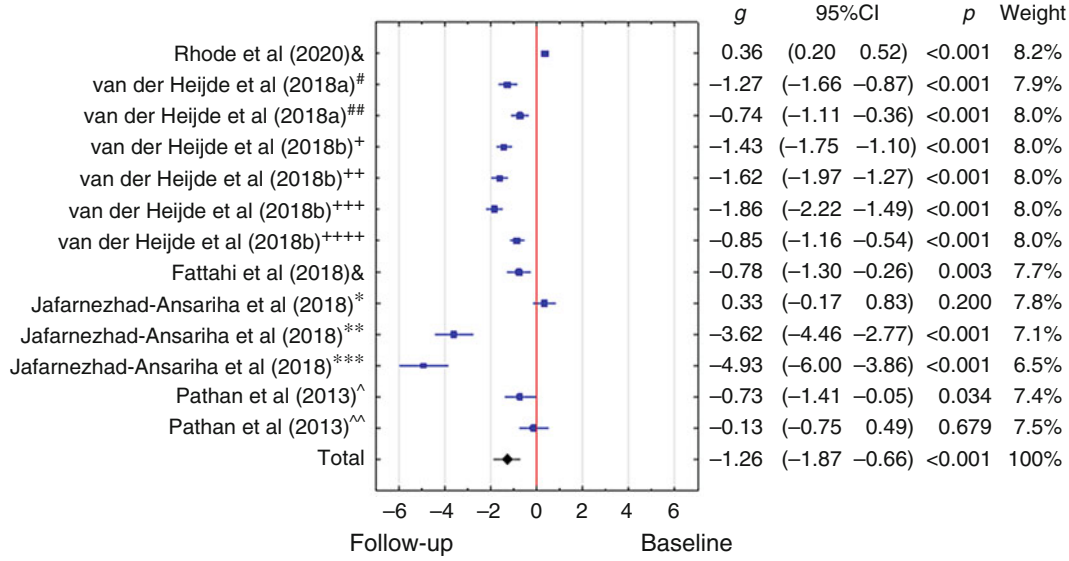

Fig. 6 Functional assessment in ankylosing spondylitis (AS) patients treated with biological and traditional drugs at baseline and treatment follow-up, based on the Bath Ankylosing Spondylitis Functional Index (BASFI) questionnaire. The random-effects model with Hedges' *g*. The effect size and confidence interval for each study appear graphically on a separate row (blue). The summary effect and its confidence interval are displayed at the bottom (black). 95% CI, lower and upper 95% confidence intervals; *filgotinib, **placebo, $^{+}$adalimumab, $^{++}$ixekizumab Q2W, $^{+++}$ixekizumab Q4W, $^{++++}$placebo, $^{\#}$β-D-mannuronic acid, $^{\#\#}$naproxen, $^{\#\#\#}$placebo, ^apremilast, ^^placebo

The evaluation of functional performance in the AS patients using the BASFI also showed, overall, significant score decreases pointing to improvements, irrespective of the therapy mode (Fig. 6). There were, however, single exceptions to the opposite. Rohde et al. (2020) noticed no improvement in the BASFI score (2.6 *vs.* 3.4; p >0.05) in a group of patients treated with biological drugs at a 5-year follow-up. Likewise, no functional improvement was noticed while using a placebo (4.7 *vs.* 5.8; p >0.05) in a study of Jafarnezhad-Ansariha et al. (2018) (Table 4).

Table 4 Functional assessment in AS patients based on the Bath Ankylosing Spondylitis Functional Index (BASFI) at baseline and follow-up after treatment, considering all drugs

Study	Treatment	Baseline		Follow-up		
		Patients (n)	Score	Months	Patients (n)	Score
Rohde et al. (2020)	Drugs unstratified	380	2.6 ±2.2	60	240	3.4 ±2.2
Van der Heijde et al. (2018a)	Filgotinib	58	7.0 ±1.5	3	58	4. 6 ±2.2
	Placebo	58	6.9 ±1.6	3	58	5.6 ±1.9
Van der Heijde et al. (2018b)	Adalimumab	90	6.1 ±2.1	4	90	4.0 ±0.2
	Ixekizumab Q2W	83	6.3 ±2.1	4	83	3.9 ±0.2
	Ixekizumab Q4W	81	6.1 ±1.8	4	81	3.7 ±0.2
	Placebo	87	6.4 ±1.9	4	87	5.2 ±0.2
Fattahi et al. (2018a)	Drugs unstratified	30	4.4 ±2.0	3	30	2.9 ±1.8
Jafarnezhad-Ansariha et al. (2018)	β-D-mannuronic acid	30	4.4 ±0.3	3	30	3.3 ±0.3
	Naproxen	28	4.2 ±0.3	3	28	2.7 ±0.3
	Placebo	27	4.7 ±0.3	3	27	5.8 ±0.3
Pathan et al. (2013)	Apremilast	17	4.6 ±2.4	3	17	2.8 ±2.2
	Placebo	19	3.5 ±2.2	3	19	3.2 ±2.0

Scores are means ±SD

Table 5 Factors affecting the quality of life (QoL) in ankylosing spondylitis (AS) patients

Factors increasing QoL	Factors decreasing QoL
Younger age, higher education, lower disease burden, low BASDAI, high BAS-G, high CRP, no use of biological treatment at baseline, and low HAQ score (Rohde et al. 2020), upadactinib 15 mg once a day (van der Heijde et al. 2019), filgotinib (van der Heijde et al. 2018a), adalimumab and ixekizumab (van der Heijde et al. 2018b), β-D-mannuronic acid, naproxen, lower CRP, and longer time elapsing from the intervention (Arısoy et al. 2013), apremilast (Pathan et al. 2013)	Anxiety and depression, erythrocyte sedimentation rate, Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) score (Arısoy et al. 2013)

3.2 Positive and Negative Predictors of Quality of Life (QoL) in Patients with Ankylosing Spondylitis (AS)

Besides QoL scoring, the research on QoL often includes the search for the presaged predictors of QoL. In the relevant publications, the following factors are listed as positive predictors: age, social support, professional activity, illness acceptance, and rehabilitation. Conversely, negative predictors are the following: living alone, depression, severe symptoms, pain, frequent hospitalizations, and old age (Jankowska-Polanska et al. 2017; Polanski et al. 2016). The present meta-analysis revealed that positive predictors for QoL among patients with AS included younger age, better education, lower symptom severity, treatment with biologicals and other selected drugs, and shorter treatment duration. The adverse predictors were anxiety and depression, and greater symptom severity. These results are summarized in Table 5.

Biological drugs are recommended for SA patients who do not respond to other drugs over 6 months' treatment. These drugs, however, have adverse events that are displayed and compared against placebo in Fig. 7. The most often in decreasing frequency are the following: infections, inclusive of upper respiratory tract infections (133 patients), neutropenia grade 1 (32 patients), headache (31 patients), and injection site reactions (25 patients). Out of these

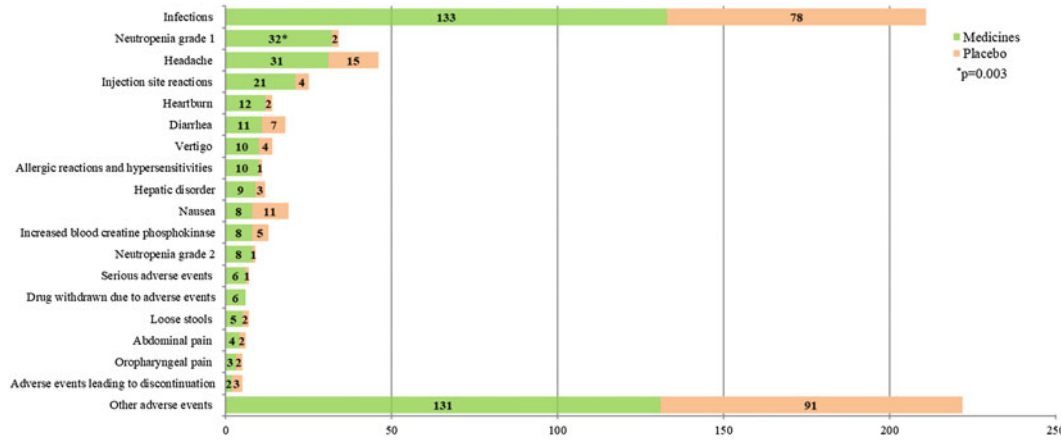

Fig. 7 Adverse effects in ankylosing spondylitis (AS) patients treated with biological drugs *versus* placebo; *significant difference between biological drugs and placebo

events, neutropenia grade 1 occurred significantly more often in patients treated with biological drugs (adaliumumab Q2W, ixekizumab Q2W, and ixekizumab Q4W) than placebo; 5.7% versus 0.6%, respectively ($p = 0.003$). The incidence of the other treatment-associated adverse events differed insignificantly between the biological drugs and placebo.

4 Discussion

According to the New York criteria, AS affects 0.007–1.7% of the global population, with an annual incidence of 0.44–7.3 *per* 100,000 individuals (Stolwijk et al. 2012). The prevalence in central Europe is estimated at 0.5% and most patients are men. It is a potentially serious disease with a variety of symptoms, typically requiring multidisciplinary treatment coordinated by a rheumatologist. Treatment for AS is complex, comprising medicines, physiotherapy, psychotherapy, and surgical interventions. The severity of chronic pain and stiffness increases gradually as the inflammation spreads to higher segments of the spine, disrupting its normal curvature. The associated ankylosis, peripheral joint deformities, and extremity contractures often lead to severe disability. As the disease often affects young people, most commonly in their thirties, it interferes with professional and social activity. About 10–30% of patients give up professional activity within 10 years of disease onset due to the progressive functional restriction. The available literature indicates that the life expectancy of AS patients is shorter than that in the general population due to disease complications (Haroon et al. 2015). This meta-analysis shows that biological drugs used in SA offer the benefit of a better patient QoL compared with the traditional non-biological treatment. Both kinds of treatments have the potential to generate a similar specter of multi-system adverse effects during a long-term follow-up. Biological drugs, noticeably, significantly more often may cause mild neutropenia than do non-biological ones. Nonetheless, the advantage of QoL with biological drugs provides a rationale to consider them as the more effective option.

The main objective of AS treatment is achieving the best possible QoL in the long term, by managing symptoms and inflammation, preventing progressive structural damage, and maintaining or restoring functional performance and ability to participate in social activities. For more than 10 years, biological drugs have been used as a pharmacological tool for AS treatment in Poland, with a lot of hope placed in these medications by physicians and patients alike. The drugs in use are monoclonal antibodies that

bind to humoral factors and cells involved in the immune response, thus inhibiting inflammation. Their application provides benefits like a rapid effect, symptom alleviation, longer periods of remission, a lower level of pain, and better QoL. Notably, QoL in terms of physical functioning is enhanced more than that of mental health (Law et al. 2018). Gorman et al. (2002) have reported that etanercept produces significant improvements in morning stiffness, spinal pain, number of swollen joints, and functional performance in AS patients. Studies on the use of TNF-α inhibitors in AS treatment also show less pain and improvements in functional activity and performance and laboratory tests, including blood CRP levels (Tłustochowicz 2011). In this meta-analysis, we also found a downward trend in CRP blood content, taken as a clinical index of treatment effectiveness. Likewise, in a study performed at the Institute of Rheumatology in Prague, Chechia, nearly one-half of AS patients treated with biological drugs had a positive and effective clinical response (Lachaine et al. 2013). In a study by van der Heijde et al. (2018b), patients treated with adalimumab showed an improvement in QoL at a 3–6-month follow-up, sustained for 5 years despite the signs of inflammation found in X-ray images. The improvement was particularly clear while using the BASFI and ASQoL questionnaires. According to those authors, the QoL is mainly determined by functional performance and disease activity. On the other side, the physical domain of QoL is affected by spine mobility and disease activity determined by the irreversible structural damage and reversible inflammation in the spine (Machado et al. 2010). Rohde et al. (2020) have found that patients with axial spondyloarthritis do not show a deterioration in health-related QoL during 5 years of treatment with biological drugs but have a significant improvement in physical health evaluated by the generic SF-36 questionnaire. In another study, the administration of 15 mg of upadacitimid once daily produced a response 2 weeks after treatment onset in SA patients, consisting of back pain reduction that lasted for 14 consecutive weeks and was confirmed by abating inflammation of the spine and sacroiliac joints in the magnetic resonance imaging (van der Heijde et al. 2019). The authors have documented that improvements in functioning and a reduction in disease activity were greater in patients treated with biological drugs than in those receiving placebo, with adverse events being of no major concern or requiring a change or discontinuation of therapy. However, patients treated with the biological drug had a higher creatinine kinase level, albeit not associated with more severe functional impairment.

In another study, the effect of filgotinib was assessed, with the primary endpoint being a change in disease activity from the baseline level (van der Heijde et al. 2018a). At a 3-month follow-up, the mean ankylosing spondylitis disease activity score (ASDAS) was higher in the filgotinib group when compared to placebo, showing the effectiveness of the drug in reducing symptoms of AS inflammation. The biological drug was well tolerated. The incidence of adverse events in the biological therapy group was akin to that in the placebo group as also was the number of patients who discontinued the treatment. In a different study by van der Heijde et al. (2018b), ixekizumab was administered every 3 or 4 weeks and its effects were compared against placebo. At a 4-month follow-up, physical function improved, and the disease activity was lower in patients treated with the biological drug, and the improvements were greater than those possibly observed with placebo. No difference was found concerning adverse events between the biological drug and placebo.

Another biological drug, proven effective in SA, is β-D-mannuronic acid (Fattahi et al. 2018a). The drug appeared effective 2 weeks after treatment onset and over 3 months of continued therapy. Likewise, improvements mainly concerned the disease activity and functional performance. This study did not compare the drug with a placebo. In another study, Jafarnezhad-Ansariha et al. (2018) have compared the therapeutic effectiveness of β-D-mannuronic acid to a combination of naproxen and placebo. The outcome measure was a mean change from baseline to therapy week 12. The authors show beneficial effects of the drug in that

it reduced pain, stiffness, and inflammation assessed by the blood CRP level, as well as improvements in physical function assessed by BASDAI and BASFI scores. Further, treatment-associated adverse events did not exceed those observed in the control treatment consisting of naproxen and placebo. Fattahi et al. (2018b) have also compared the therapeutic effectiveness of β-D-mannuronic against naproxen with placebo and confirmed good tolerance and high effectiveness of the biological drug over a 3-month follow-up. Beneficial effects included less pain, better functional performance, and lower CRP levels. In this study, the biological drug was associated with fewer adverse events compared to naproxen. On the other hand, Pathan et al. (2013) have investigated the treatment with an oral phosphodiesterase 4 inhibitor, apremilast, on the premise that it might be effective and well-tolerated in AS patients as it modulates biomarkers of bone biology. The study, however, has failed to establish the presence of appreciable benefits at a 3-month follow-up compared to placebo.

The known effects of biological drugs are not limited to the inhibition of disease progression, improvements in functional status, and reductions in stiffness, pain, and inflammation. Studies have shown the effectiveness of TNF-α inhibitors, e.g., infliximab, in the treatment of depressive symptoms accompanying the AS (Arısoy et al. 2013). In a study by Ertenli et al. (2012), infusions of infliximab were associated with a gradual reduction of depression and anxiety symptoms and QoL improvements since the very beginning of the intervention, and the drug's effectiveness increased with consecutive infusions.

Among predictors of QoL, the literature notably mentions higher education associating with a better long-term health-related QoL (Kotsis et al. 2014). The relation of patient age to the QoL remains somehow debatable. A younger age often means a shorter duration of illness, and thus, less structural damage and fewer complications (Rohde et al. 2020). On the other hand, the duration of illness is difficult to establish in patients with axial SA as symptoms may develop for up to 10 years before the diagnosis is made (Feldtkeller et al. 2000).

A major limitation of this meta-analysis is a lack of standardized data in all papers included in it, which concerns the research instruments used, intervention duration, and inclusion or not of a control group. Moreover, the scoring of instruments is not always compatible with each other in different papers, which hampers the interpretation of findings. Another limitation is the use of different active substances and different follow-up periods across the studies. Additionally, some studies included the assessment at just two-time points whereas repeated assessments at multiple equal intervals would have produced clearer results.

5 Conclusions

We conclude that patients suffering from ankylosing spondylitis, generally, benefit from regular treatment concerning QoL and functional performance. The benefits are greater with biological therapy than with using a placebo. The QoL is positively influenced by younger age, higher education, better functional status, biological therapy, and a longer of treatment, and negatively influenced by anxiety and depression. There appears no appreciable difference concerning the adverse events between biological and standard therapy, except for the propensity for neutropenia that is more common in the biologically treated patients. To achieve a well-scrutinized view on ankylosing spondylitis treatment, it is recommendable that future evaluations be based on studies that involve the controlled use of the same drug and employ the same standardized research instruments. Nonetheless, we believe that the finding that biological drugs are superior in improving the QoL should strengthen the recommendations for their use in patients with AS.

Conflicts of Interest The authors declare no conflicts of interest in relation to this article.

Ethical Approval This review article does not contain any studies with human participants or animals performed by any of the authors.

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Changes of Motile Ciliary Phenotype in Patients with Primary Ciliopathies

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Abstract

Primary ciliopathies are a group of disorders associated with abnormal formation and function of primary cilia. Many cilia-associated proteins found in primary cilia are also present in motile cilia. Such proteins are important for the ciliary base, such as the transition zone or basal bodies, and the intraflagellar transport. Their exact role in the respiratory motile cilia is unsettled. In this prospective clinical single-center study, we investigated the hypothesis that these proteins regulate the function of motile cilia. We addressed the issue by defining the motile cilia beat frequency in the respiratory tract of patients with primary ciliopathies accompanied by chronic kidney disease and comparing it in those without kidney involvement. Ciliary beat frequency in the nasal mucosa samples was evaluated by the ciliary analysis software LabVIEW. Both children and their parents with primary ciliopathies and kidney involvement had significantly lower median airway ciliary beat frequencies than those without kidney involvement who have normal ciliary motility. Further, the ciliary beat frequency is inversely associated with the serum creatinine level. These findings strongly suggest that kidney involvement in patients with primary ciliopathy may underlie the development of motile cilia dysfunction in the respiratory tract, potentially increasing respiratory morbidity.

Keywords

Cilia · Ciliary beat frequency · Ciliopathy · Kidney disease · Respiratory morbidity

1 Introduction

Cilia play a vital part in human physiology. They are divided into two main types. Motile cilia are found in the respiratory tract, ventricles of the central nervous system, and the middle ear. These cilia have a rhythmic beating motion and, for instance, in the respiratory tract, they are an important component of the airway defense by sweeping mucous out of the lungs (Kempeneers and Chilvers 2018). Primary cilia are nonmotile and typically appear on the apical surface of most eukaryotic cells as microtubule-based organelles covered with the plasma membrane. These cilia lack the two central microtubule singlets

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characteristic of motile cilia. Primary cilia play an essential role for chemosensory (Bloodgood 2010), mechanosensory, for instance, detecting luminal flow in renal tubules, biliary ducts, and vessels (Nauli et al. 2003), and photosensory retinal functions (Senior et al. 1961). Primary ciliopathies are a group of disorders associated with abnormal formation and function of primary cilia. The clinical phenotypes of ciliopathy are diverse. A common feature of some primary ciliopathies is a variable extent of kidney damage or cyst formation (Braun and Hildebrandt 2017). Typical extra-renal manifestations include hepatic cysts and fibrosis (Schueler et al. 2015), retinal degeneration (Senior et al. 1961), skeletal deformities, dysmorphic facial features (Sensenbrenner et al. 1975), laterality defects, and congenital heart defects (Otto et al. 2003). Respiratory manifestations of primary ciliopathies like bronchiectasis, respiratory infection, or lung cysts are rare and their exact cause is unclear (Ibrahim and Rasoul 2015; Driscoll et al. 2008). Nonetheless, the respiratory tract is quite often affected by the disturbance of motile cilia manifest by impaired function of mucociliary clearance. The ciliary beating is coordinated in metachronal waves. It is a complex process that requires intertwined molecular interactions influenced by intracellular Ca^{2+} content and pH change, and protein kinase activation (Joskova et al. 2020; Marusiakova et al. 2020; Bayless et al. 2019; Duricek et al. 2019). Many cilia-associated proteins found in primary cilia are also present in motile cilia. Yet it is unclear to what extent proteins forming the ciliary basal body and the transition zone, and those engaged in the intraflagellar transport, could be concurrently affected in both types of cilia. Therefore, this study aims to evaluate the ciliary beat frequency (CBF) of the airway motile cilia in patients with a primary ciliopathy in an organ distal to the respiratory tract. We investigated the hypothesis that the airway CBF could be affected in primary ciliopathies involving kidneys, which would manifest in reduced airway mucus clearance. We addressed the issue by investigating the pediatric and adult (children's parents) patients with primary ciliopathies affecting kidneys.

2 Methods

2.1 Study Design and Population

This is a prospective clinical single-center study. We had four groups of patients: two each of children and adults with and without chronic kidney diseases, CKD(+) and CKD(−), respectively. Additionally, we had two groups of healthy age-matched children and adults with no kidney disease for the reference measurement of CBF. The group stratification with basic demographic data is shown in Table 1.

Inclusion criteria were the lack of acute respiratory infections in the preceding 4 weeks, no smoking, no allergy, and no use of antihistamines. The degree of chronic kidney disease (CKD) in adults was evaluated according to the National Kidney Foundation's CKD Epidemiology Collaboration (CKD-EPI) creatinine equation (Levey and Stevens 2010) and in children according to the bedside Schwartz equation (Schwartz and Work 2009). Basic demographic data, detailed respiratory history, laboratory indices data (plasma creatinine, urinalysis), abdominal ultrasonography, echocardiography, spirometry, and chest computer tomography were obtained from medical records. Exclusion criteria were adenoid hypertrophy, acute or chronic infections, and anatomical deformities identified during the ear-nose-throat examination.

Table 1 Stratification of study groups

	Primary ciliopathy—children		Primary ciliopathy—adults		Healthy children	Healthy adults
	CKD(+)	CKD(−)	CKD(+)	CKD(−)	CKD(−)	CKD(−)
n	3	16	3	5	43	21
Age (years)	14 (12–16)	9 (6–11)	54 (37–59)	37 (35–43)	10 (7–13)	42 (34–49)

Data are medians (IQR). *CDK(+)* chronic kidney disease, *CKD(−)* lack of chronic kidney disease, *n* number of subjects

Other exclusion criteria were gastroesophageal reflux disease, endocrine, metabolic, or oncological diseases.

2.2 Investigations

Samples of ciliated epithelium in nasal mucosa were obtained by a cytology brush (Cytobrush plus, Medscand Medical; CooperSurgical, Inc., Trumbull, CT) and suspended in the Roswell Park Memorial Institute (RPMI) 1640 medium. The temperature was maintained at 20 °C. Motile cilia were observed using a digital high-speed video camera (Basler A504kc; Basler AG; Ahrensburg, Germany) at a frame rate of 256–512 per sec. The camera was connected to an inverted phase-contrast microscope (Zeiss Axio Vert. A1; Carl Zeiss AG: Göttingen, Germany) and a computer. Specimens were examined using a 40× objective lens providing a magnification of 400×. Video analysis was performed using cilia analysis software (LabVIEW National Instruments Corp, Austin, TX) (Hargaš et al. 2011). Each preparation was evaluated for a maximum of 10 min, with 10 short video sequences recorded. The median and range of CBF were provided. Only undisrupted ciliated epithelia were analysed.

Genetic analysis was performed to confirm the presence of primary ciliopathy using the next-generation sequencing method using NextSeq System (Illumina Inc., San Diego, CA). The entire coding and splice-relevant regions of genes were evaluated.

2.3 Statistical Analysis

Data were expressed as medians and interquartile ranges (IQR). Differences were assessed using Student's *t*-test for unpaired samples. A *p*-value <0.05 defined statistically significant changes. The analysis was performed using a commercial SYSTAT v11.0 package (Systat Software Inc., San Jose, CA).

3 Results

3.1 Airway Ciliary Beat Frequency (CBF)—Motile Phenotype

The airway CBF in healthy children and their adult parents without primary ciliopathy and chronic kidney diseases was, on average, between 6 and 8 Hz and was alike in both groups as detailed in Table 2. This value of CBF was taken as a reference level for comparison to that in patients with primary ciliopathies and chronic kidney involvement. Children and their parents with CKD had the median airway CBF, on average, between 4 and 5 Hz, which was a highly significant decline compared to the patients without CKD ($p < 0.001$). There were no appreciable differences in CBF between the two age groups of patients in the presence or absence of CKD (Table 3). The CBF in patients without CKD was at the level of that present in the control healthy subjects.

Table 2 Airway ciliary beat frequency in healthy control subjects without primary ciliopathy and chronic kidney diseases (CKD−)

	CKD(−)
Ciliary beat frequency (Hz)	
Children	6.7 (6.0–7.7)
Adults	6.2 (5.3–7.3)
p (children/adult)	> 0.05
Creatinine (μmol/l)	
Children	55 (47–66)
Adults	73 (67–79)
p (children/adult)	0.03

Data are medians (IQR)

Table 3 Airway ciliary beat frequency and serum creatinine level in patients with and without chronic kidney diseases, CKD(+) and CKD(−), respectively

	CKD (+)	CKD (−)	*p*
Ciliary beat frequency (Hz)			
Children	4.0 (3.9–4.8)	6.8 (6.5–7.1)	< 0.0001
Adults	4.9 (4.3–5.0)	6.9 (6.7–7.1)	< 0.001
p (children/adult)	0.45	0.39	
Creatinine (μmol/l)			
Children	296 (225–299)	57 (47–78)	0.00003
Adults	216 (186–315)	62 (47–81)	0.003
p (children/adult)	0.43	0.39	

Data are medians (IQR)

3.2 Respiratory Manifestations

Respiratory manifestations were seen in 100% of children with CKD(+) and 6.3% of children with CKD(−). Concerning the adult patients, respiratory manifestations were seen in 33.3% with CKD(+) and in none with CKD(−). Patients with nephronophthisis type I (NPHP type 1) did not suffer from respiratory infections but had a restrictive lung disorder and nonspecific nodules up to 5 mm in diameter in the upper lobes of lung parenchyma (segments: S10 bilateral and S6 in the right lung). Overall, a restrictive lung disorder was seen in 50% of patients with CKD. A 10-year-old boy with Sensenbrenner syndrome (SBS) and low CBF had mild recurrent rhinosinusitis. Two patients with autosomal recessive polycystic kidney disease (ARPKD) had recurrent rhinitis. One of them belonged to the CKD(+) group and had a strongly decreased lower CBF of 3.8 (3.2–6.0) Hz. Another CKD(+) patient with the autosomal dominant polycystic kidney disease (ADPKD) and a restrictive lung disorder also had a low CBF of 3.8 (3.2–5.9) Hz. Computer tomography of the lungs did not detect bronchiectasis in the patients.

3.3 Genetic Analysis

The presence of primary kidney ciliopathy was confirmed in the genetic analysis. We found that patients with ADPKD commonly had a mutation in the *PKD1* gene (88.0%). We identified 6 new pathogenic variants of this gene found in 11 patients (Table 4).

4 Discussion

This study demonstrates that patients with primary ciliopathies with developed CKD had reduced ciliary beating frequency in the airway mucosal cells. The most likely underlying reason could be an impaired ciliary structure caused by mutations of the genes encoding ciliary proteins that are identical for both primary and motile cilia. Several hypotheses of the origin of respiratory manifestations in patients with primary kidney ciliopathies are presented in the literature. One of them is a disorder of calcium homeostasis leading to reduced cellular calcium signaling in patients with polycystic kidney diseases. Calcium plays a critical role in regulating CBF (Mangolini et al. 2016; Nauli et al. 2003; Braiman and Priel 2001). Wu et al. (2009) have suggested that damage to primary cilia in airway smooth muscle cells could underlie motile cilia dysfunction. Bronchiectases are frequently associated with irregular bronchial wall thickness. Primary cilia of airway smooth muscle cells may be mechanical pressure sensors and play a role in cell migration, injury repair, and possibly in ciliogenesis. Motile ciliated cells originate from primary ciliated cells. When ciliogenesis is impaired, cilia exhibit slow and dyskinetic motion (Jain et al. 2010). An unresolved question is whether the sensory function of primary cilia is also present in motile cilia (Jain et al. 2012).

The present findings are comparable to those of Shoemark et al. (2015) who have found that patients with Bardet-Biedl syndrome have normal CBF. Likewise, Fliegauf et al. (2006) have found that in the absence of nephrocystin, a molecular

Table 4 Pathogenic mutations in the *PKD1* gene that encodes the protein polycystin-1 active in kidney primary cilia. Individual subjects, renal and extrarenal manifestations, as well as the patients' airway ciliary beat frequency (CBF) are shown. New pathogenic mutations unraveled are bold out

Sex	Mutation/gene	CBF (Hz)	Renal manifestations	Extrarenal manifestations
F	*PKHD1*	3.8	CKD G4, HT, P	Hepatic cysts and fibrosis, respiratory infections
M	*PKHD1*	6.5	HT	Hepatic cysts, respiratory infections
F	*PKHD1*	7.1		
F	*PKHD1*	6.0	HT	Hepatic fibrosis
F	***PKD1* c.2528C > G, p.(Ser843*) exon 11**	9.0		
M	***PKD1* c.7525_7539dup, p.(Val2509_Leu2513dup)**	8.3		
M	***PKD1* c.11425G > C, p.(Gly3809_Arg)**	8.7		
F	*PKD1*	7.0		
M	***PKD1* c.3114delA, p.(Leu1039*)**	7.0		
M	***PKD1* c.3400_3401delArg(p.Ser1134)**	6.4		
M	***PKD1* c.3400_3401delArg(p.Ser1134)**	6.9		
F	***PKD1* c9683dupG, p.(Leu3229Profs*24) exon 28**	6.7		
F	*PKD2*	6.6		
F	*PKD2*	7.2		
F	*PKD1*	3.0	CKD G4, HT, P	Hepatic cysts, restrictive lung disorder
F	*PKD1*	4.9	CKD G3b, HT, P	Hepatic cysts
M	*PKD1*	5.0	CKD, G3a, HT	
M	***PKD1* c.3400_3401delAG(p.Ser1134)**	6.7	HT	
M	*PKD1*	7.1		
F	*PKD2*	6.2	HT, P	Hepatic cysts
F	*PKD1*	6.9		
M	***PKD1* c.3114delA, p.(Leu1039*)**	7.9		
F	*NPHP1 (homozygote)*	4.0	CKD G5, P, HT, Tx	LVNC, nonspecific lung nodules, restrictive lung disorder
M	***WDR35* c.1922 T > G, p.(Leu641*), c.2522A > Tp.(Asp841Val)**	5.6	CKD G3b, HT, P	Foramen ovale, hypospadias, Cranioectodermal dysplasia, respiratory infections
M	***WDR35* c.1922 T > G, p.(Leu641*), c.2522A > Tp.(Asp841Val)**	6.5		Cranioectodermal dysplasia
M	*BBS9*	6.7		Syndactyly, Hirschsprung disease, hypogonadism
F	OFD type I	6.5		Syndactyly, brachydactyly

CKD chronic kidney disease, *Arg* arginine, *Asp* aspartate, *BBS* Bardet-Biedl syndrome, *Gly* glycine, *HT* hypertension, *Leu* leucine, *LVNC* left ventricular non-compaction cardiomyopathy, *OFD type I* orofaciodigital syndrome, *P* proteinuria, *Ser* serine, *Tx* kidney transplantation, *Val* valine

component of primary cilia, the structure of motile cilia remains unaltered, and the CBF is normal. The beating pattern may be slightly irregular, but the irregularity is not as severe as in primary ciliary dyskinesia. Therefore, the presumption that proteins that regulate ciliary motion would be damaged in patients with primary ciliopathies is hardly tenable.

Ciliopathies are a heterogeneous group of disorders. Phenotypic features are variable even in patients with the same mutations. Walczak-Sztulpa et al. (2017) have reported the intrafamilial variability in patients with Sensenbrenner syndrome with heterozygous variants in the *WDR35* gene. We observed two female siblings with ciliopathies having different phenotypic features (these two patients were not included in the present study group; unpublished observation). One girl had polycystic kidney disease. Her renal function was normal and the CBF remained in the normal range of 7.2 (6.5–8.4) Hz. The other girl had the CBF significantly reduced down to the level of 1.1 (0–2) Hz characteristic of primary ciliary dyskinesia. This girl had a normal renal function and no cystic kidney formations but suffered from recurrent respiratory infections. The plausibility is that the siblings had different clinical manifestations of the same cilia-elated disorder. However, the varied expression of relevant genes or genetic mutations ("genetic load") that can modulate clinical manifestation cannot be excluded (Arts and Knoers 2013; Bredrup et al. 2011).

It is generally known that patients with CKD are more likely to experience respiratory manifestations (Pierson 2006). A cross-sectional study of Kucur et al. (2016) who used saccharin tests suggests that chronic kidney failure is a risk for respiratory mucociliary dysfunction. According to that study, nasal mucociliary clearance time is significantly greater in patients with CKD than in healthy control individuals. No studies have yet directly evaluated the respiratory CBF in patients with kidney failure. There are several potential mechanisms of damage to respiratory cilia in this condition like free oxygen radicals or uremic toxins, endothelial dysfunction and vasoconstriction with reduced blood flow, decreased periciliary fluid flow, or increased mucus viscosity with electrolyte imbalance (Uluyol et al. 2016). The design of the present study did not make it possible to distinguish the exact molecular mechanism of respiratory cilia dysfunction. Yet we believe we have shown that motile cilia in the airways of patients with CKD are defunct as expressed by significantly reduced CBF. Further, we submit that genetic factors and disturbed body homeostasis in CKD may impair mucociliary transport function in the airways of patients with primary ciliopathies.

Conflicts of Interest The authors declare no conflicts of interest in relation to this study.

Ethical Approval All procedures performed in studies involving human participants were in accord with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent Informed consent was obtained from all individual participants included in the study.

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Effects of Inhalation of STIM-Orai Antagonist SKF 96365 on Ovalbumin-Induced Airway Remodeling in Guinea Pigs

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Abstract

Airway remodeling (AR) consists of wall thickening and hyperreactivity. STIM (stromal interaction molecule) and Orai protein pathways mediate extracellular Ca^{2+} signals involved in AR. This study aims to define the effects on AR of the STIM-Orai antagonist SKF 96365 given by inhalation in three increasing doses in ovalbumin-induced AR. In the control group, the antiasthmatic budesonide and salbutamol were given in the same model. The airway structure was evaluated by histological and immunohistochemistry and reactivity by specific airway resistance, contraction strength of isolated airway smooth muscles, and mucociliary clearance expressed by ciliary beating frequency. The immuno-biochemical markers of chronic inflammation were evaluated by BioPlex and ELISA assays. The AR was mediated by inflammatory cytokines and growth factors. The findings show significant anti-remodeling effects of SKF 96365, which were associated with a decrease in airway hyperreactivity. The anti-remodeling effect of SKF 96365 was mediated via the suppression of IL-4, IL-5, and IL-13 synthesis, and IL-12–INF-γ–TGF-β pathway. The budesonide-related AR suppression had to do with a decrease in proinflammatory cytokines and an increase in the anti-inflammatory IL-10, with negligible influence on growth factors synthesis and mucous glands activity.

Keywords

Airway hyperactivity · Airway remodeling · Biomarkers · Cytokines · Inflammation · SKF 96365 · STIM-Orai pathway

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1 Introduction

Airway remodeling (AR) refers to structural changes resulting from repeated injury and repair processes. The AR in asthma consists of epithelial alterations and metaplasia, increased deposition of extracellular matrix components under the bronchial subepithelial basement membrane (Boulet 2018), airway smooth muscle (ASM) hypertrophy and hyperplasia (Munakata 2006), gland enlargement, and neovascularization (Bergeron et al. 2010). Immunologic and molecular mechanisms that drive the progression of asthma to AR are still incompletely understood. Studies of allergen-induced AR in transgenic mice suggest roles for cytokines, chemokines, and growth factors, such as transforming growth factor-beta (TGF-β), vascular endothelial growth factor (VEGF), Th_2 cytokines—interleukin-5 (IL-5), IL-9, and IL-13, and epithelial-derived nuclear factor kappa B (NF-κB)-regulated chemokines (Doherty and Broide 2007). The release of TGF-β by injured epithelium results in the synthesis of extracellular matrix proteins by fibroblasts and myofibroblasts (Hostettler et al. 2008) and secretion of cytokines, chemokines, and growth factors by modulating autocrine proliferative responses of ASM, all of which driving the AR (Spinelli et al. 2012; Shore 2002). Some other cytokines affecting airway inflammation or structural cells such as tumor necrosis factor-alpha (TNF-α), IL-4, fibroblast growth factor (FGF), or epidermal growth factor (EGF) also have this potential (Busse et al. 2005; Kuperman et al. 2005).

Growth factors acting via tyrosine kinase receptors, inflammatory mediators, like histamine, acting via G protein-coupled receptors, and cytokines acting via cell surface glycoprotein receptors increase the cytoplasmic content of calcium ions (Ca^{2+}). A rise in Ca^{2+}, besides contraction and proliferation of ASM, mediates mucus production, ciliary beat frequency, or cytokine/chemokine synthesis in structural and inflammatory cells. A major conduit for Ca^{2+} influx in non-excitable cells is through plasma membrane store-operated channels (SOC) (Samanta and Parekh 2016) following the emptying of Ca^{2+} stores in the endoplasmic reticulum. In immune cells, calcium release-activated channels (CRAC), containing stromal interaction molecule (STIM) and Orai proteins in the endoplasmic reticulum, activate de novo synthesis and secretion of pro-inflammatory leukotrienes and increase expressions of c-Fos, nuclear factor of activated T-cells (NFAT), and various cytokines which orchestrate the subsequent inflammatory responses (Sutovska et al. 2016; Hogan et al. 2010; Vig et al. 2008). SOC-mediated Ca^{2+} entry also is involved in TGF-β-facilitated ASM cell proliferation (Gao et al. 2013) and IL-13/TNF-α induced airway hyperresponsiveness (Spinelli and Trebak 2016; Jia et al. 2013). The ASM remodeling in asthma is associated with changes in the expression of ion channels and pumps (Mahn et al. 2010; Perez-Zoghbi et al. 2009). The ASM cells from asthmatic mice display an increased expression of STIM-Orai proteins (Spinelli et al. 2012).

Targeting CRAC channels seems a promising approach to managing chronic airway diseases. The combined modulation of airway epithelial, ASM, and inflammatory cell activities by CRAC channel inhibitors could effectively dampen airway hyperreactivity, inflammatory, and remodeling components. We investigated this hypothesis in the present study in an experimental model of ovalbumin-induced airway remodeling in the guinea pig.

2 Methods

2.1 Animals

A total of 70 adult guinea pigs, strain TRIK, weighing 150–300 g were used in the study. The animals were obtained from the Department of Experimental Pharmacology of the Slovak Academy of Sciences, in Dobra Voda, Slovakia, and underwent at least one-week adaptation period in the animal house, having commercial chow and water ad libitum. Additionally, they were adapted to the laboratory environment for

several days before experiments, with the ambient temperature of 21–24 °C and relative humidity of 55 ± 10%. There were 7 randomly selected groups consisting of 10 animals each. The airway inflammation was induced in six groups, with the remaining group serving as a healthy ovalbumin-negative (OVA-) control. All the remaining animals were sensitized by repetitive administration of the OVA allergen for 28 days. From the 14th day of sensitization on, the treatment by salmeterol, budesonide, and SKF 96365 (1-[2-(4-Methoxyphenyl)-2-[3-(4-methoxyphenyl) propoxy]ethyl-1*H*-imidazole hydrochloride) was started in five experimental groups. The sixth group, OVA28, was exposed only to isotonic saline (5 min inhalations) and served as a sensitized negative control group. SKF 96365 groups were exposed for 5 min to different doses/concentrations of the compound by the inhalation route: SKF2.5 – 2.5 μM, SKF5.0 – 5.0 μM, and SKF10 – 10 μM. The tested SKF 96365 compound and two control drugs, salmeterol (SAL – 0.17 mM, 5 min inhalation) and budesonide (BUD – 1 mM, 5 min inhalation), were applied once daily for 14 days. The influence of SKF 96365 on airway defense mechanisms was evaluated in all three concentration groups receiving it. The immunohistochemistry was performed on specimens taken from the group of animals treated with the highest SKF 96365 concentration of 10 μM having the most pronounced effects.

2.2 Reagents

Chicken ovalbumin, histamine, salmeterol, and budesonide were purchased from Sigma-Aldrich (Bratislava, Slovakia), SKF 96365 from Tocris Bioscience (Bristol, Great Britain), aluminum hydroxide and Tween 80 from Centralchem (Bratislava, Slovakia), methacholine and saline (sodium chloride solution) from ApliChem (Darmstadt, Germany), RPMI 1640 medium from Gibco/Thermo Fisher Scientific (Waltham, MA).

SKF 96365 and salmeterol were dissolved in water for injection, budesonide in 1% Tween 80, and all other chemicals in saline. Solutions of all compounds were aerosolized by a PARI jet nebulizer (output 5 L/s, particles mass median diameter 1.2 μm; Paul Ritzau, Pari-Werk GmbH, Starnberg, Germany) and delivered to the head-out plethysmograph composed of separate nasal and body chambers (HSE type 855; Hugo Sachs Electronic, March, Germany) where the animals were placed. The experimental procedures were accomplished 24 h after the last drug application.

2.3 Ovalbumin Sensitization and Challenge Protocol

For the 28-day long sensitization, OVA adsorbed on aluminum hydroxide in saline, 5 mg OVA, and 100 mg $Al(OH)_3$ was administered parenterally as follows: Day 1 intraperitoneally and subcutaneously, Day 4 intraperitoneally, and Day 12 subcutaneously. Afterward, it was nebulized and the mist was introduced into the head chamber of a plethysmograph at Days 9, 15, 18, 20, 24, and 27. The OVA(−) group of animals (healthy negative control) was challenged for 28 days with isotonic saline only.

2.4 Morphology and Immunohistochemistry

Airway Reactivity In Vivo Specific airway resistance (sRaw) was used to evaluate the airway reactivity in vivo, mostly expressing the contractility of ASM cells. Conscious guinea pigs were individually placed in the head-out body plethysmograph. sRaw was calculated according to Pennock et al.'s (1979) method based on the airflow difference between the two chambers of the body plethysmograph. Changes in sRaw were recorded for 1 min after 30-s long histamine (10^{-5} M) and methacholine (10^{-5} M) inhalation. There was a 1 min interval between inhalations of bronchoconstrictor, with fresh air blowing into the head chamber.

Ciliary Beat Frequency (CBF) In Vitro A specimen of the ciliated epithelium was brush-taken from the trachea for an in vitro investigation. The

temperature of the nutritive RPMI 1640 medium for cilia was maintained at 37–38 °C using a PeCon Temp Controller 2000–2 (PeCon GmbH; Erbach, Germany). Specimens were put onto glass slides and evaluated under an inverted phase-contrast microscope (Zeiss Axio Vert. A1; Carl Zeiss AG; Jena, Germany). Impaired ciliated cells were discarded. Sequential 10-s video files were recorded at 1 min intervals for 15 min at a frame rate from 256 to 512 frames/s using a digital high-speed video camera (Basler A504kc; Basler AG; Ahrensburg, Germany). The CBF was evaluated using Ciliary Analysis software (LabVIEW™) to generate a ciliary region of interest (ROI) (Hargas et al. 2011).

Histological and Immunohistochemical (IHC) Analysis Histomorphological and IHC staining was performed on formalin-fixed paraffin-embedded tissue samples collected from guinea pigs' left lungs, serially cut at 4 μm thickness. Gömöri's staining was used for reticulin fibers, anti-alpha smooth muscle actin (SMA), and anti-mucin 5 AC (MUC5AC) antibodies (Abcam; Cambridge, UK) for immunohistochemistry. To achieve a better adherence of tissue sections for immunoreactions, we used silanized slides (DAKO; Glostrup, Denmark), which were baked for 2 h in an oven at 59 °C. Immunohistochemical staining was performed by the autostainer BenchMark ULTRA (Roche; Rotkreuz, Switzerland) with mouse monoclonal antibodies MUC5AC (MRQ 19 clone, the incubation time of 16 min) and SMA (1A4 clone, the incubation time of 28 min) using Kit ultraView DAB (Ventana Medical Systems Inc.; Munich, Germany). All sections were counterstained with hematoxylin (DAKO, Glostrup, Denmark). For negative controls, the primary antibody was omitted. Slides were viewed under the Olympus BX41 microscope (Olympus; Tokyo, Japan). The image capture and the measurement of the thickness of bronchi walls (μm) in 10–15 similar size random bronchi in each section were performed using Quick Photo Micro software v2.2 (Olympus, Tokyo, Japan). The degree of MUC5AC positivity was semi-quantitatively determined by two independent observers under a dual-head microscope and grading system as previously described (Sutovska et al. 2015). Each specimen processed was evaluated as negative (degree 0 and 1) or positive (degree 2 and 3).

2.5 Biochemistry

Cytokines Animals were killed by transversal interruption of the cervical spinal cord and respiratory tract organs were removed. Bronchoalveolar lavage (BALF) was obtained by application of 10 mL/kg of 0.9% saline at 37 °C BBC Breaking News @BBCBreaking· Mar 4 Prince Philip has had "successful procedure" for pre-existing heart condition and will stay in hospital for number of days, Buckingham Palace says into a ligated left lung. The supernatant was acquired by centrifugation at 1500 rpm for 2 min. The Bio-Plex® 200 System and Bio-Plex Pro™ Human Cytokine Th1/Th2 Assay (Bio-Rad, Hercules, CA) were used to assess the content of cytokines and chemokines involved with the allergic inflammation of airways, such as IL-4, IL-5, IL-10, IL-12, IL-13, TNF-α, granulocyte-macrophage colony-stimulating factor (GM-CSF), and interferon-gamma (INF-γ). The assay is based on the sandwich ELISA design using magnetic beads. The measured analyte is bound between the capture and detection antibodies. The capture antibody-coupled beads were first incubated with antigen standards, samples, or controls, followed by the incubation with biotinylated detection antibodies and the reporter streptavidin-phycoerythrin (S-P) conjugate. Then, the beads were passed through the Bio-Plex 200 suspension array reader, equipped with two lasers of 532 nm and 635 nm excitations to measure the fluorescence of beads and bound S-P. A high-speed digital processor managed the data output, and the Bio-Plex Manager™ v6.0 software presented the concentration results in pg/mL.

Enzyme-Linked Immunosorbent Assay (ELISA) and Specific Reagents Supernatants from BALF and lung tissue homogenates were used for the quantification of growth factors. Lung tissue homogenates were prepared by sonication with a power output of 700 W (Stuart homogenizer in

SHM/STAND; Ecomed, Bratislava, Slovakia) in 1 mL of lysis solution (Tissue Extraction Reagent I Invitrogen™, ThermoFisher Scientific, Waltham, MA) containing a protease inhibitor cocktail (Sigma Aldrich Chemicals, St. Louis, MO). Samples were centrifuged at 10,000 rpm for 5 min at 4 °C (MICRO 220R Centrifuge, Hettich GmbH, Tuttlingen, Germany). BALF and homogenate supernatants were collected into sterile tubes and frozen at −80 °C for further analysis.

The level of TGF-β was determined in the supernatant from lung homogenates using an ELISA kit for transforming growth factor beta-1 (USCN Life Science Inc., Houston, TX) and EGF in the BALF supernatant using a kit for guinea pig total epidermal growth factor (MyBioSource, San Diego, CA) according to the manufacturer's instructions. The absorbance was measured spectrophotometrically at a wavelength of 450 nm and output data were assessed by SkanIt Software for Varioskan® Flash v2.4.5 (ThermoFisher Scientific, Waltham, MA). The detection levels were 5.7 pg /mL for TGF-β and 0.1 ng/mL for EGF.

2.6 Statistical Elaboration

Data on cytokines, growth factors, and sRaw were expressed as means ±SE. Data on the thickness of the SMA/collagen III layer were expressed as medians of representative 12–15 bronchial walls assessed in each guinea pig. CBFs were expressed as a median value (Hz) for each ROI, followed by the calculation of the arithmetic mean in each microscopic preparation. Student's *t*-test or one-way ANOVA with *post-hoc* Bonferroni test was selected as appropriate to test for statistically significant intergroup differences. Fisher's exact test was selected to evaluate the findings in the immunohistochemical analysis of MUC5AC. A p-value <0.05 defined significant differences. The analysis was performed using a commercial statistical package of GraphPad Prism software v6.01 (San Diego, CA).

3 Results

3.1 Effects of SKF 96365 on Airway Defense Mechanisms

Specific Airway Resistance (sRaw) Consistent with the histomorphological part of the study that clearly showed an increase in the ASM mass in the OVA28 group, there were significant differences in the basal, histamine-induced, and methacholine-induced sRaw when compared to OVA(−) group (Fig. 1). In vivo contractility of airways, whether in basal conditions or induced by either bronchoconstrictor, was significantly reduced in all experimental, with SKF 96365, and positive control, with salmeterol, groups of guinea pigs. SKF 96365 is a nonspecific SOC inhibitor causing effective and relatively selective CRAC channel inhibition when it is used in a concentration from 2 μM (Singh et al. 2010) to 12 μM (Spinelli and Trebak 2016; Chung et al. 1994), and salmeterol is a well-known classic asthma reliever. The CRAC channel blocker showed a dose-dependent suppression of mediator-induced airway hyperreactivity. Further, 14-day-long daily inhalation of 10 μM SKF 96365 exceeded the impact of the long-acting β-agonist salmeterol.

Ciliary Beating Frequency (CBF) We assessed the CBF as a key determinant of inflammation-induced AR in the ovalbumin-sensitized animals and the influence of CRAC channel inhibition on mucociliary clearance. The AR was associated with a negligible decrease in CBF. However, long-term treatment by SKF 96365 in the lowest tested concentration of 2.5 μM significantly inhibited the motion of cilia, as opposed to the control drugs budesonide and salmeterol or SKF 96365 in higher doses, all of which did not further suppress the CBF (Fig. 2).

Morphological Features of Airways Immunohistology was used to evaluate lung tissue specimens for the inflammation-induced structural changes in the ovalbumin-sensitized animals and the subsequent effects of long-term treatment

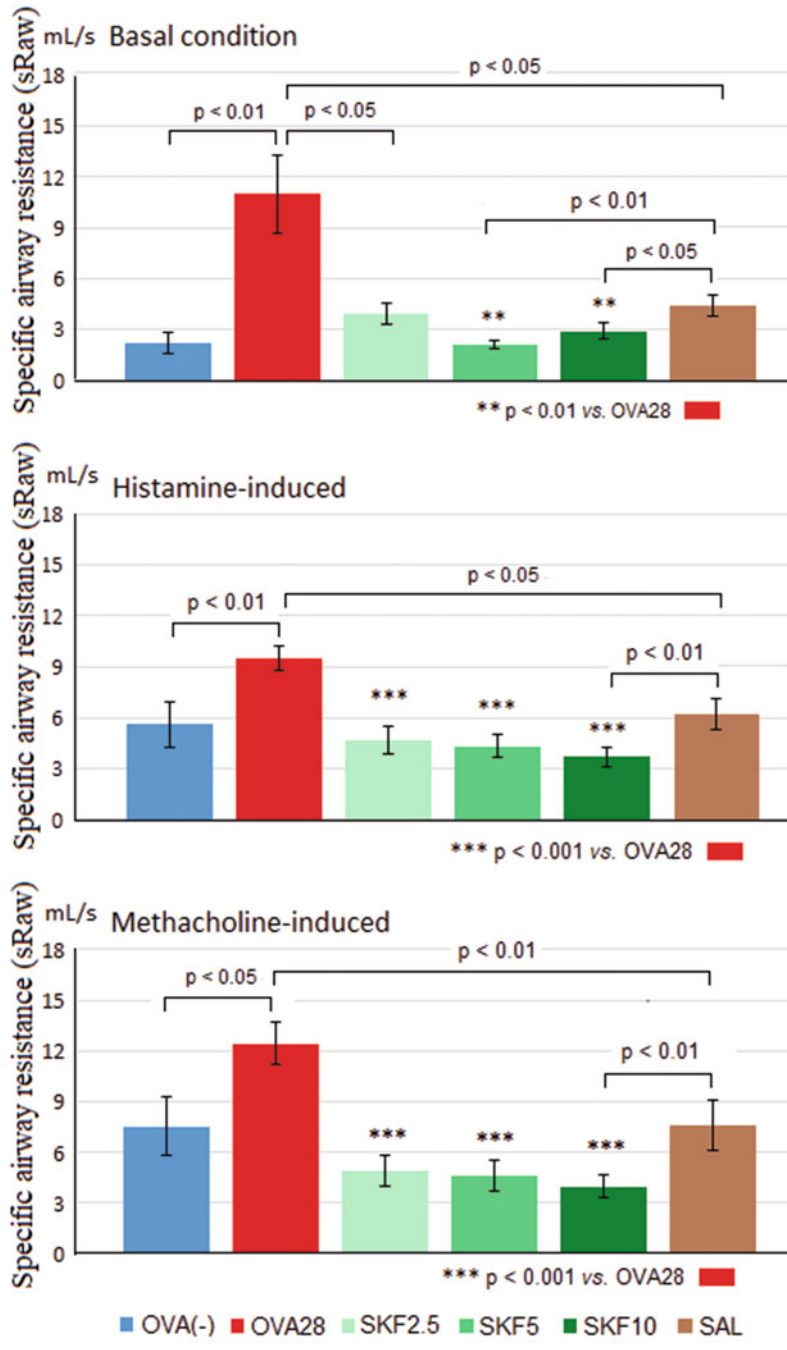

Fig. 1 Effects of inhalation of SKF 96365 in increasing concentrations on airway hyperreactivity assessed from the contractile response on the background of the ovalbumin (OVA)-sensitized guinea pigs. Top panel—basal condition—OVA sensitization and inhalations of isotonic saline only; middle panel—histamine inhalation in OVA-sensitized animals; bottom panel—methacholine inhalation in OVA-sensitized animals. The contractile response was expressed as the specific airway resistance (sRaw). OVA(−), unsensitized control; OVA28, sensitized negative control group; SKF2.5, 5, and 10 sensitized and treated with inhalation of SKF 96365 in the successively doubled concentrations of 2.5, 5, and 10 μM; and SAL, sensitized positive control group treated with inhalation of beta-2 agonist salmeterol (one-way ANOVA and post hoc Bonferroni test)

by the highest concentration of SKF 96365 as well as the classical corticosteroid anti-asthmatic budesonide. For comparison, specimens from saline-exposed animals were used (OVA-). Specimens from the sensitized negative controls (OVA28) showed the remodeling features such as subepithelial fibrosis, goblet cell hyperplasia, and increased ASM mass (Fig. 3). These changes were partially reverted in specimens from both SKF 96365 and budesonide-treated animals.

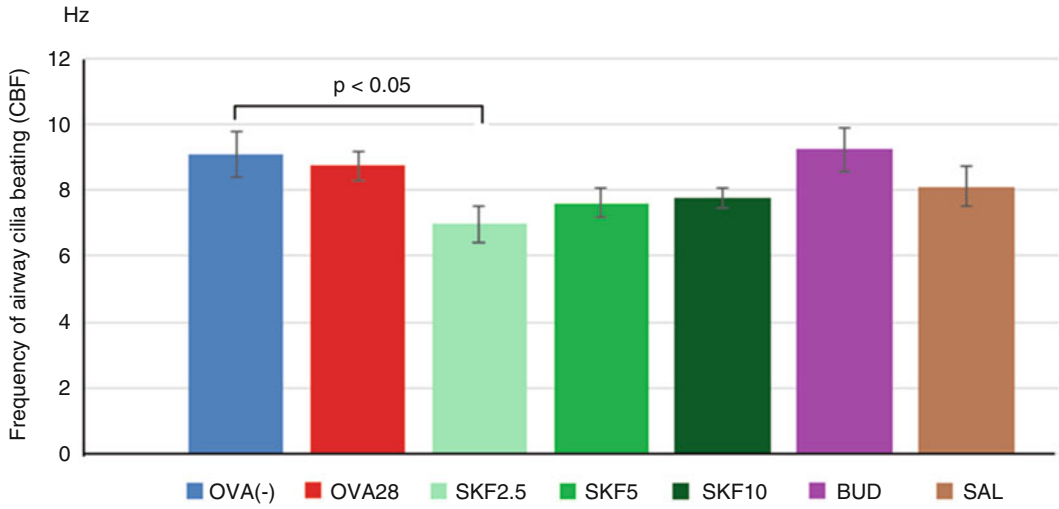

Fig. 2 Cilia beating frequency (CBF) in the investigated groups of guinea pigs: OVA(−), unsensitized control group; OVA28, ovalbumin-sensitized negative control group treated with isotonic saline; SKF2.5, 5, and 10, sensitized and treated with inhalation of SKF 96365 in the successively doubled concentrations of 2.5, 5, and 10 μM; and BUD and SAL, sensitized positive control groups treated with inhalations of budesonide and salmeterol, respectively (one-way ANOVA and post hoc Bonferroni test)

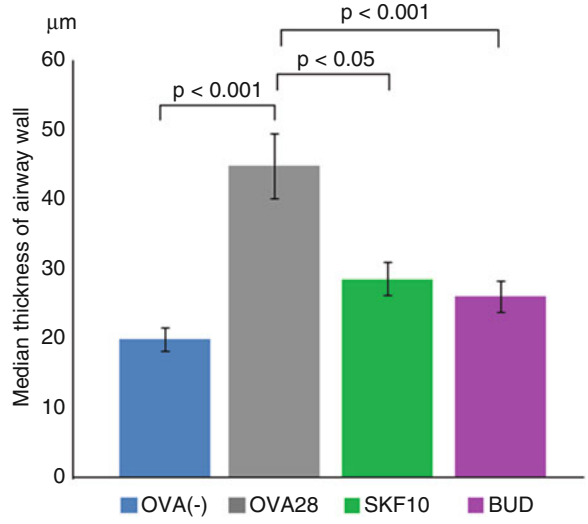

Fig. 3 Thickness of smooth muscle actin (SMA) in airway walls visualized with monoclonal SMA (1A4 clone) antibody and chromogen (3,3′-diaminobenzidine). OVA (−), unsensitized guinea pigs; OVA28, ovalbumin-sensitized negative control group treated with isotonic saline; SKF10, sensitized and treated with inhalations of 10 μM SKF 96365; and BUD, sensitized positive control group treated with budesonide (see Methods for details) (one-way ANOVA and post hoc Bonferroni test)

Specimens from the OVA(−) animals showed no signs of remodeling. Likewise, immunohistochemistry for SMA (Fig. 4) and MUC5AC showed significant expression enhancements in the sensitized negative controls (OVA28). Only was the SKF 96365 capable of reducing these AR

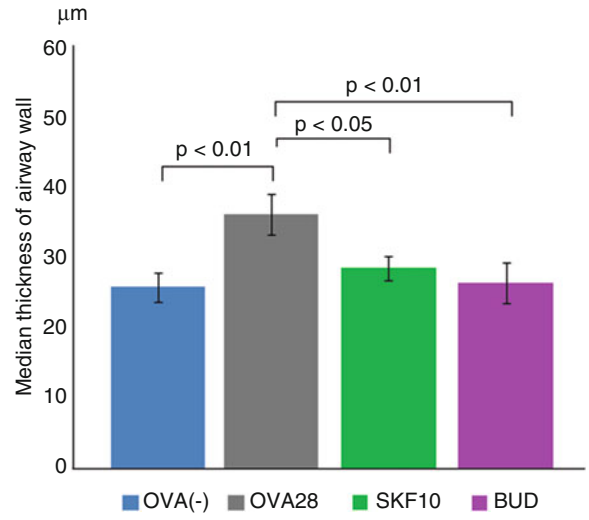

Fig. 4 Thickness of reticulin fibers (collagen III) in airway walls visualized with Gömöri's staining. OVA(−), unsensitized guinea pigs; OVA28, ovalbumin-sensitized negative control group treated with isotonic saline; SKF10, sensitized and treated with inhalation of 10 µM SKF 96365; and BUD, sensitized positive control group treated with budesonide (one-way ANOVA and post hoc Bonferroni test)

Table 1 Semi-quantitative analysis of anti-mucin 5 AC (MUC5AC) antibody positivity. The number of negative (infiltration score 0 and 1) and positive (infiltration score 2 and 3) samples in ovalbumin-unsensitized – OVA(−) guinea pigs, ovalbumin-sensitized negative controls treated with isotonic saline (OVA28), and ovalbumin-sensitized guinea pigs treated with inhalation of SKF 96365 in a concentration of 10 µM (SKF10), and ovalbumin-sensitized positive controls treated with inhalation of budesonide (BUD)

	Negative samples	Positive samples
OVA(−)	4	4
OVA28	0	10*
SKF10	5	3**
BUD	3	6

*p < 0.05 versus OVA(−); **p < 0.01 versus OVA28 (Fisher's exact test)

features, including goblet cell hyperplasia and overproduction of mucin. Budesonide effectively suppressed ASM hyperplasia and prevented fibrotic changes but failed to influence the MUC5AC enhancement (Table 1).

3.2 Cytokines and Growth Factors

Cytokine Content To gain insight into the mechanisms of AR, we quantitatively evaluated the content of key cytokines and growth factors in the lung tissue. IL-2, IL-4, IL-5, IL-10, IL-12 (p70), IL-13, TNF-α, GM-CSF, and INF-γ were examined in control OVA-sensitized untreated and treated with SKF 96365 and anti-inflammatory budesonide. IL-4, IL-5, IL-13, and IL-10 were significantly elevated in BALF collected from sensitized negative controls (OVA28) when compared to healthy unsensitized animals. SKF 96365 and budesonide significantly reduced the content of cytokines characteristic for chronic allergic inflammation and AR, such as IL-4, IL-5, and IL-13. Further, SKF 96365 enhanced the synthesis of the anti-inflammatory IL-12 and INF-γ. Unlike the SKF 96365, budesonide failed to affect the synthesis of IL-12 and INF-γ but induced a release of the anti-inflammatory IL-10. This effect, however, was significant only when compared to that in OVA(−) but not sensitized negative controls (OVA28) (Fig. 5).

Epidermal Growth Factor (EGF) and Transforming Growth Factor-Beta (TGF-β) The lung tissue EGF and TGF-β were significantly elevated after 28-day long sensitization (Fig. 6). Only SKF 96365 treatment was capable of reducing the synthesis and release of TGF-β,

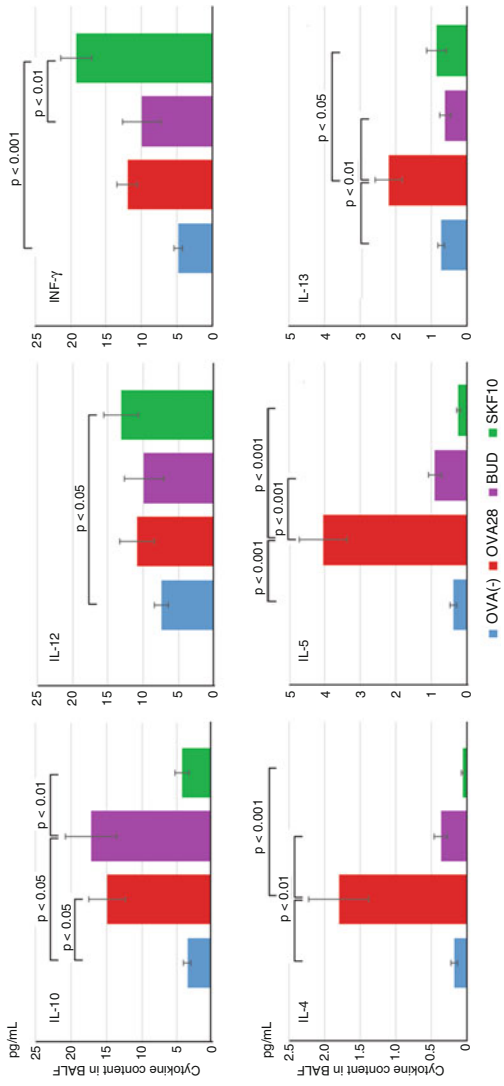

Fig. 5 Cytokine content in BALF. IL, interleukins, INF-γ, interferon-gamma; OVA (−), unsensitized guinea pigs; OVA28, ovalbumin-sensitized negative control group treated with isotonic saline; SKF10, sensitized and treated with inhalation of 10 μM SKF 96365; and BUD, sensitized positive control group treated with budesonide (one-way ANOVA and post hoc Bonferroni test)

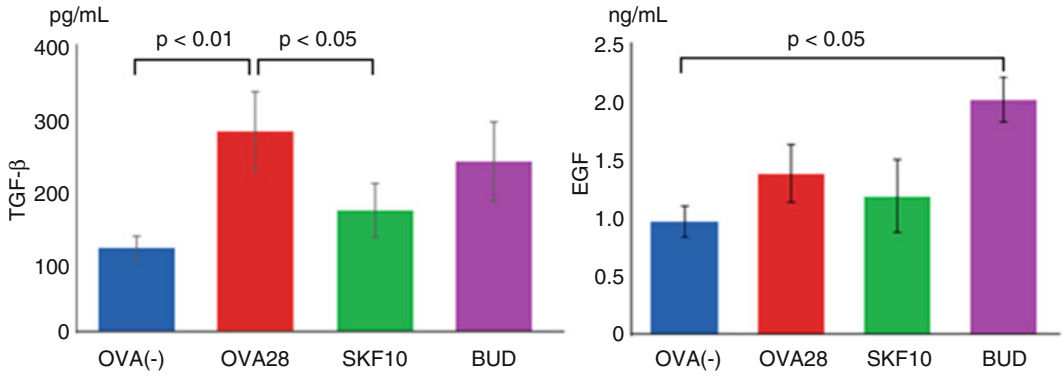

Fig. 6 Growth factors engaged in airway remodeling: transforming growth factor-beta (TGF-ß) and epidermal growth factor (EGF), evaluated in the supernatant of lung tissue homogenate. OVA(−), unsensitized guinea pigs; OVA28, ovalbumin-sensitized negative control group treated with isotonic saline; SKF10, sensitized and treated with inhalation of 10 μM SKF 96365; and BUD, sensitized positive control group treated with budesonide (one-way ANOVA and post hoc Bonferroni test)

which corresponded to an increase in IL-12 and INF-γ (BioPlex analysis), confirming the activation of the IL-12–INF-γ–TGF-β anti-remodeling pathway. However, SKF 96365 failed to significantly affect the EGF content that remained elevated. Budesonide failed to reduce either TGF-β or EGF. Since TGF-β is believed to be causally associated with goblet cells hyperplasia and mucus hyperproduction in asthma, these results are in line with the histomorphological findings outlined above.

4 Discussion

This study investigated whether blocking the CRAC channel activity could retard the airway remodeling (AR) induced on the background of chronic allergic inflammation induced by ovalbumin (OVA) sensitization in the guinea pig. The guinea pig model of allergic inflammation closely resembles the asthmatic process in the human airways. Allergen sensitization and repeated aerosolized allergen challenges are the accepted way of inducing AR, which is routinely assessed by histological examination of paraffin-embedded specimens of the airway tissue (McGovern and Mazzone 2014). Although the challenges are recommended for 12 weeks, the modified 28-day long model of chronic inflammation we used in the present study showed key AR features such as mucus hypersecretion evidenced by the enhanced MUC5AC protein expression, subepithelial fibrosis evidenced by an increase in the mass of reticulin fibers in Gömöri's staining, and ASM hyperplasia evidenced by the enhanced expression of SMA in bronchial walls. Further, we found a close association of histomorphological features of AR with the impaired airway defense mechanisms and immuno-biochemical changes, pointing to fully developed allergic inflammation, a major sign of which was a significant increase in in vivo basal and bronchoconstrictor-induced hyperreactivity-sensitized animals compared to healthy controls challenged with saline only. The CBF, a key predictor of mucociliary clearance, decreased. The AR in our model corresponded to the increased content of markers of remodeling such as the volume of elastic fibers and actin in the airway and lung tissue exposed to cumulative doses of OVA twice a week for 4 weeks (Pigati et al. 2015) or and to allergic rhinitis induced by repeated OVA intraperitoneal administration followed by intranasal challenges within 3 weeks (Chen et al. 2017) in the guinea pigs. These changes also correspond well to the epithelial airway dysfunction typical of AR in

chronic asthma reported in humans where subepithelial fibrosis and mucus hypersecretion commonly result in impaired mucociliary clearance (Burgess et al. 2016; Sedaghat et al. 2016; Borish 2002; Durrani et al. 2011; Al-Muhsen et al. 2011).

In the present study, the content of IL-4, IL-5, IL-10, and IL-13 increased in BALF after 28-day long OVA sensitization in healthy guinea pigs. The IL-13, particularly, is one of the most crucial mediators of allergic inflammation. There is evidence that blocking the IL-13 in the airways alone is sufficient to prevent airway inflammation, hyperreactivity, ciliated cell loss, and mucus overproduction induced by allergen challenges in animal models of asthma (Erle and Sheppard 2014; Ford et al. 2001). IL-13-induced stimulation of the airway epithelium activates hypersecretory MUC5AC-expressing mucus cells. IL-4 appears to have effects akin to those of IL-13, as both share the common receptor on club cell, type II IL-4R whose signaling plays a role in allergen-induced mucus hyperproduction (Kuperman et al. 2005). ASM cells in asthmatic airways proliferate in response to IL-4, IL-13, and IL-5 which is a key activator of eosinophils (Halwani et al. 2010; Doherty and Broide 2007). Contrarily, the synthesis of IL-10, a prototype anti-inflammatory cytokine, decreases in asthmatic airways (Jahromi et al. 2014; Ogawa et al. 2008). Unlike the clinical condition, where the development of asthma is often associated with the immune-related predisposition, in the present experimental study we used healthy guinea pigs and repeatedly exposed them to the OVA allergen mimic allergic airway inflammation and AR. The premise was that OVA would induce chronic airway inflammatory and remodeling changes, without much immune involvement. In fact, the stimulation of protective mechanisms driven by IL-10 and IL-12 in OVA28 animals, appearing with AR, was statistically insignificant when compared to the OVA(−) group. On the other hand, 10 μM concentration of SKF 96365 not only decreased the content of the asthma-related proinflammatory cytokines production IL-4, IL-5, and IL-13 but also significantly countered the increase in the anti-inflammatory IL-12–IFN-γ pathway. The Th1 cytokines IL-12 and IFN-γ form a natural counterbalance to Th2 cytokines, driving protective cell-mediated immunity (Biedermann et al. 2004). IFN-γ secreted by Th1 cells activates macrophages and dendritic cells to produce IL-12 which, in turn, decreases the antigen-induced bronchial hyperresponsiveness, eosinophilia, and mucus goblet cell hyperplasia induced by Th2 cells (Barnes 2008; Caramori et al. 2008).

Airway structural changes are variably influenced by Th2 cytokines and growth factors. TGF-β, produced in large quantities by immune cells, fibroblasts, and epithelial cells, regulates the proliferation of immune cells and their recruitment into tissues (Bush 2019; Pakyari et al. 2013). Prochazkova et al. (2012) have shown that IL-12 is a cytokine having the ability to skew the ongoing TGF-β-dependent differentiation into Th1-like direction. IL-12 and IFN-γ production in asthmatics is often on the low side. The IL-12–INF-γ axis is considered a key downstream pathway in Th2-dependent asthma (Kim et al. 2010). Noteworthy, IL-12 activity may be countered by IL-4 action (Wong et al. 2001).

In the present study, AR features got retracted during the long-term administration of SKF 96365 in the concentration needed to selectively inhibit CRAC channels. The corticosteroid budesonide, tested in parallel for comparison, significantly inhibited ASM proliferation and increase in reticulin fiber mass but was unable to reduce mucus hypersecretion by the airway epithelium. This finding is consistent with literature data that steroids have limited effects on airway mucus hypersecretion (Shen et al. 2012). SKF 96365 exhibited similar or greater suppression of airway hyperreactivity than the classic bronchodilator salmeterol and, except for the lowest concentration tested, bronchodilatory effects were not accompanied by an adverse influence of airway cilia. Increases in the anti-inflammatory IL-12 and INF-γ caused by SKF 96365, a CRAC channel blocker, points to the possible mechanism of its anti-remodeling effect. Jia et al. (2013) have shown that mainly IL-13 enhances cytoplasmic puncta formation of ectopically

expressed fluorescently tagged STIM1 and increased store-operated Ca^{2+} entry (SOCE) in ASM cells, suggesting that proinflammatory cytokines might contribute via CRAC channels to airway hyperresponsiveness. In line with this suggestion, Jairaman et al. (2016) have provided evidence that bronchial epithelial cells sense some allergens through the activation of cell surface protease-activated receptor type 2, which leads to the opening of store-operated CRAC channels. These channels are thus posed to have a central role in allergen signaling in the airway epithelium.

Corticosteroid drugs are essential anti-inflammatory drugs in the treatment of asthma. In a guinea pig asthma model of Pigati et al. (2015), dexamethasone inhibited the secretion of the pro-inflammatory Th2 cytokines IL-4, IL-5, IL-13, and TNF-α, as well as INF-γ. In this study, we used budesonide as a positive control drug and noticed the effects on cytokines in BALF akin to those exerted by dexamethasone above quoted. Moreover, budesonide tended to increase the content of IL-10, which could have a mitigating influence on AR.

The present findings also show that TGF-β and EGF increased in BALF from the OVA28 group of animals, which corresponded to histomorphological alterations. It is generally accepted that TGF-β plays a regulatory role in AR. The evidence to support the contribution of TGF-β to AR in asthma is derived from several murine and human studies (Miller et al. 2006; Flood-Page et al. 2003). TGF-β induces the apoptosis of airway epithelial cells and increases goblet cell proliferation, which suggests its role in mucus hyperproduction. TGF-β also increases fibroblast proliferation with their differentiation into myofibroblasts and the synthesis of extracellular matrix protein in subepithelial fibrosis facilitating ASM contractility and airway narrowing (Ojiaku et al. 2017; Makinde et al. 2007). Further, agonists of airway bronchoconstriction, like methacholine, enhance the release of TGF-β and other mediators from ASM cells, which augment airway narrowing and remodeling (Oenema et al. 2013; Grainge et al. 2011). Thus, TGF-β represents a bonding link for airway remodeling and hyperreactivity in asthma. The finding of the present study is that SKF 96365, inhibiting the STIM-Orai pathway, but not budesonide, significantly decreased TGF-β content in BALF. This result is in line with the study of Gao et al. (2013) where TGF-β promoted the rat ASM cell proliferation increasing STIM-Orai1 and SOCE activities, which was partially reduced by SKF-96365. While corticosteroid therapy reduces airway inflammation in asthma, the role of glucocorticoids concerning TGF-β synthesis is contentious. Some murine studies show that dexamethasone, budesonide, and fluticasone inhibit TGF-α (Takami et al. 2012) but fail to affect TGF-β (Halwani et al. 2011), while others show the anti-remodeling effect of corticosteroids mediated through a decrease in TGF-β expression (Doherty and Broide 2007; Miller et al. 2006; McMillan et al. 2005). No effects on TGF-β or inhibition of collagen deposition and mucus hyperproduction in human asthma have yet been reported (Chakir et al. 2003).

The EGF upregulates MUC5AC gene transcription by acting as a ligand for the epithelial growth factor receptor (EGFR) (Chen et al. 2016). Histamine and TNF-α act in airways by enhancing the EGFR-induced goblet cell hyperplasia (Hirota et al. 2012; Nadel 2001). The EGFR pathway ultimately activates the nuclear factor (NF)-κB, elevating the expression of genes encoding proinflammatory cytokines. The EGF also stimulates ASM hyperplasia and subepithelial fibrosis. Asthmatic airways show an increase in EGF and EGFR immunoreactivity not only in the bronchial epithelium but also in the airway glands, smooth muscles, and the thickened basement membrane (Amishima et al. 1998). Samanta et al. (2014) have shown that Ca^{2+} entry through Orai protein in human bronchial epithelial cells stimulates EGF gene expression together with c-fos and NFAT transcription factors as well as NFAT-driven gene expression. The activation of c-Fos and NFAT pathways is prevented by pre-exposure of bronchial epithelial cells to the CRAC channel blockers Synta66 and 3,5-bis-trifluoromethyl-pyrazole (BPT2). However, detectable basal EGF transcription is present in the absence of Orai-STIM pathway

stimulation, which fits into the known physiological role of EGF in airway function. In the present study, neither budesonide nor SKF 96365 significantly inhibited the synthesis and release of EGF in the OVA-challenged guinea pig, the content of which was close to the level present in healthy non-sensitized animals. There is a biological plausibility that the EGFR-signaling pathway, but not EGF synthesis per se, is inhibited.

In conclusion, a 28-day-long OVA sensitization followed by inhalation challenges in the guinea pig is a valid model of asthma-like airway remodeling, in which the proinflammatory cytokines IL-4, IL-5, and IL-13, as well as TGF-ß and EGF, are involved. The structural airway alterations combined with the inflammatory process related to the magnitude of airway hyperactivity, expressed by increases in basal and constriction-induced sRaw and dysfunctional mucociliary clearance. The findings indicate that corticosteroids might have a mitigating role in remodeling, possibly through increased synthesis of IL-10. Further, we identified CRAC channels, forming an essential route of Ca^{2+} entry into airway epithelial cells, as a driving force of chronic inflammation, airway hyperactivity, and remodeling based on the reversal of the changes by the administration of a specific CRACK inhibitor, SKF 96365, in OVA-sensitized animals. The IL-12–INF-γ–TGF-β pathway activation is liable to be the underlying molecular mechanism suppressing the development of airway remodeling in response to chronic inflammation. Thus, targeting CRAC channels might offer promise as a novel therapeutic approach for managing chronic airway inflammatory disorders including advanced asthma and possibly also chronic obstructive pulmonary disease.

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Conflicts of Interest The authors declare no conflicts of interest concerning this article.

Ethical Approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. The animals were treated in accord with the Guide for the Care and Use of Laboratory Animals (8th edition, National Academies Press) and the EU and Slovakian legislation regulating the welfare of experimental animals. This study was approved by a local Ethics Committee of the Jessenius Faculty of Medicine in Martin, Slovakia (permit EK 40/2018), registered by the Institutional Review Board/Ethics Board Office.

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In Silico Analysis of Bone Tension During Fixation of the Medial Malleolus Fracture After Ankle Joint Endoprosthesis

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Abstract

Total ankle arthroplasty (TAR) is a procedure alternative to arthrodesis which enables the biomechanical stabilization of the ankle joint. The procedure is associated with a high risk of complications, including fractures of the medial malleolus. In this study, the finite element method (FEM), based on CT examinations, was used to model the ankle fracture fixation after TAR. Three types of fracture stabilization were considered: screw, Blount staple, and both screw and Blount staple. In the *in silico* model, the maximum stress tension was found at prosthetic junctions with the base, cone, and talar components of the tibial prosthesis. When the fracture was stabilized with the Blount staple, tension along the cone of the tibial component was about 12% of the maximum tension. Stabilizations with the screw alone or Blount staple combined with a screw on the medial side of the cone induced tension in the immediate vicinity twice as high. In the area of the medial malleolus, the tension was alike for both types of stabilization. The tension was lowest when using the Blount staple alone. We conclude that, contrary to the hitherto clinical routine of using screws, fracture fixation using the Blount staple leads to the lowest bone tension around the fixation of the medial malleolus fracture after ankle joint endoprosthesis.

Keywords

Ankle arthroplasty · Ankle joint · *In silico* · Finite elements · Periprosthetic fracture · Talocrural joint

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1 Introduction

Total ankle arthroplasty (TAR) is a procedure more and more frequently performed. Contrary to the hip and knee joints arthroplasty, the reason for its implementation is usually not primary, but secondary to post-traumatic degenerative changes. The TAR is an alternative to arthrodesis that disrupts the proper functioning of the articular chain in the lower limb and impairs the entire

biomechanics of the locomotor system (Morash et al. 2017; Basques et al. 2016). The currently used third-generation ankle prostheses provide better biomechanical fit and survival of endoprostheses than the previous generations, although it is not the only factor determining survival (Zafar et al. 2020). Among many factors qualifying and disqualifying to the TAR, particular attention is paid to the lack of vascular impairments in the operated limb. From the biomechanical standpoint and to avoid perioperative complications, good bone stock is a key factor (Van der Plaat and Haverkamp 2017).

Ankle arthroplasty procedures, although provide better biomechanical results when compared to arthrodesis, show a significantly higher percentage of complications that mostly depend on the learning curve that describes the progress in the surgeon's experience in performing the procedure (Morash et al. 2017; Basques et al. 2016). One of the complications often arising during the implantation of an ankle joint endoprosthesis is a periprosthetic fracture. At present, the number of intraoperative fractures is decreasing but postoperative fractures are on the rise (Haendlmayer et al. 2009). The most common fractures occurring during endoprosthesis implantation concern the medial or lateral malleolus. They usually result from the use of oscillating saws and accidental cuts of the ankle or from oversizing the endoprosthesis. The reported incidence of periprosthetic fractures varies among studies. Manegold et al. (2013) have reported the incidence of 2.2%, whereas Lee et al. (2013) put the incidence of medial malleolus fractures at 10% and lateral malleolus fractures at 0.6%. In a study by Clough et al. (2018), intraoperative medial fractures accounted for 9.7% of all ankle arthroplasty procedures performed. Zaidi et al. (2013) have also reported that medial malleolus fractures as the commonest complication of arthroplasty, with an incidence of 6% compared to 1% for the lateral malleolus. It appears that as the number of procedures increases, the incidence of intraoperative fractures declines (Basques et al. 2016). Nevertheless, the issue of periprosthetic fractures in the case of TAR remains clinically significant as it affects up to 10% of patients, which makes it essential to search for the optimal method of fracture stabilization.

The finite element method (FEM) is increasingly used to define the biomechanics of fracture stabilization (Jiang et al. 2019; Anwar et al. 2017). The method enables prompt computer-based modeling and visualization of biomechanical tensions at the ankle periprosthetic fracture (Lorkowski et al. 2015, 2020). The present study aimed to create an *in silico* model of tensions arising in the medial malleolus and endoprosthesis itself using different types of fracture stabilization. Such modeling could help clarify the unresolved issue of which type of stabilization in use carries the lowest risk of implant destabilization and failure of the entire ankle arthroplasty.

2 Methods

We developed an *in silico* model of the ankle joint after arthroplasty, specifically targeted at a fracture of the medial ankle. The model was based on three clinical cases. The modeled prosthesis was of the third generation, consisting of a tibial component with a cone, a talar component made of metal alloys, and a polyethylene insert. The evaluation of bone tension arising at the bone-endoprosthesis interface transversely passing through the medial malleolus was compared between the following methods of periprosthetic fracture stabilization:

- Cortical screw passing through the medial malleolus from its apex to the shaft of the tibia, transversely through the fracture gap
- Blount staple inserted with its tines above and below the fracture gap in the medial malleolus, stabilizing the fracture externally on the medial surface of the malleolus
- Combination of these two types of stabilization, i.e., a cortical screw and Blount staple.

The bone tension, arising after fracture stabilization, was compared to that generated at the bone-endoprosthesis interface without fracture. For modeling, we used computer tomography

(CT) images of the ankle joint of an exemplary patient with an implanted joint endoprosthesis. The images, presented as a bitmap in 256 shades of gray, were processed using the software-based image registration technique to quantify tension-induced metabolic bone changes on CT images, which enables the assigning of material features to each shade of gray in the image. The resultant tension displacements at the bone-implant interface were then elaborated using the FEM 2D modeling in the ANSYS software (Ansys Inc. 2010), which enables the evaluation of tension appearing in the mechanics of solids under the influence of various factors. The area FEM-tested is divided into simple finite elements with specific geometric shapes (triangles) connected by several points at the edges, forming "knots". After selecting the nodal functions that determine the distribution of the tested physical quantity within the finite elements, calculations in the differential equations enable the determination of knots' behavior while for other points they remain approximate. After introducing the boundary conditions for the system of equations used, it is possible to determine the type and degree of displacement in nodes, which reflects tension changes present in individual parts of the bone-implant system and fracture stabilization elements (Lorkowski et al. 2020).

Based on the X-ray images, a 2D model of the ankle joint tissues and the implanted prosthesis was prepared. Overall, 16 components were obtained visible on the contour map. The images show the components representing a particular type of tissue-like bones, articular cartilage, soft elements, parts of the endoprosthesis, and elements used to fracture stabilization (Fig. 1). Young's modulus, equal to the longitudinal stress divided by the strain, and Poisson's ratio, described as a ratio of the transverse strain in the direction perpendicular to the applied force and axial strain in the direction of the applied force, were calculated for individual tissues and stabilization elements (Table 1). The former is a measure of elasticity or the ability of a bone to resist changes in length when under longitudinal tension and the latter is the ability of a bone to expand in the direction perpendicular to the direction of compression. The transverse fracture gap in the medial malleolus was modeled and then, computer models of various types of fracture stabilization were made. The models included the fracture stabilization with a screw passing through the fracture gap along the long axis of

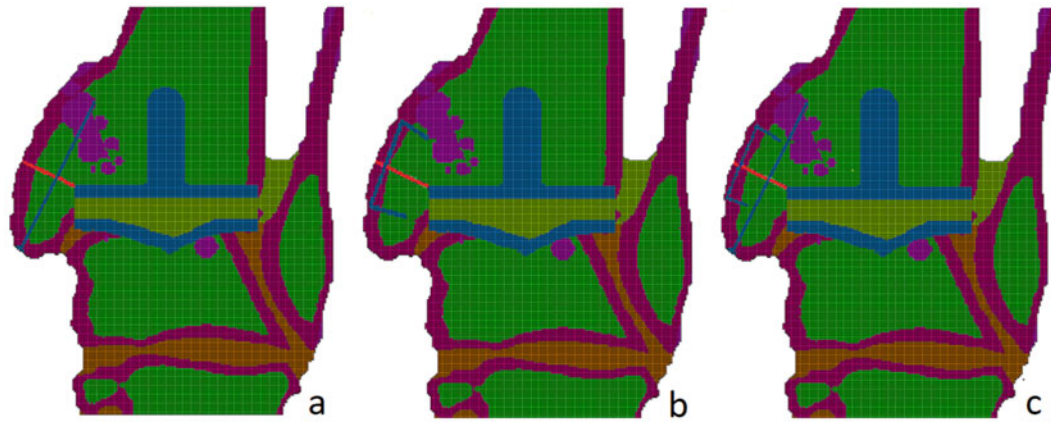

Fig. 1 A 2D ankle tissue model with implanted endoprosthesis and the stabilization elements of the medial malleolus fracture. Fracture gap marked by a red stroke on the left-hand side: (**a**) cortical screw passing through the malleolus from its apex to the shaft of the tibia, transversely through the fracture gap; (**b**) Blount staple inserted with its tines above and below the fracture gap in the medial malleolus, stabilizing the fracture externally on the medial surface of the malleolus; (**c**) combination of the cortical screw and Blount staple

Table 1 Young's modulus and Poisson's coefficient of individual tissues and elements of endoprostheses used for ankle fracture stabilization

Material	Young's modulus	Poisson's coefficient
Bone	5000 MPa	0.32
Cartilage	150 MPa	0.42
Soft tissues	800 MPa	0.42
Implant	210 GPa	0.32
Stabilization components	210 GPa	0.32

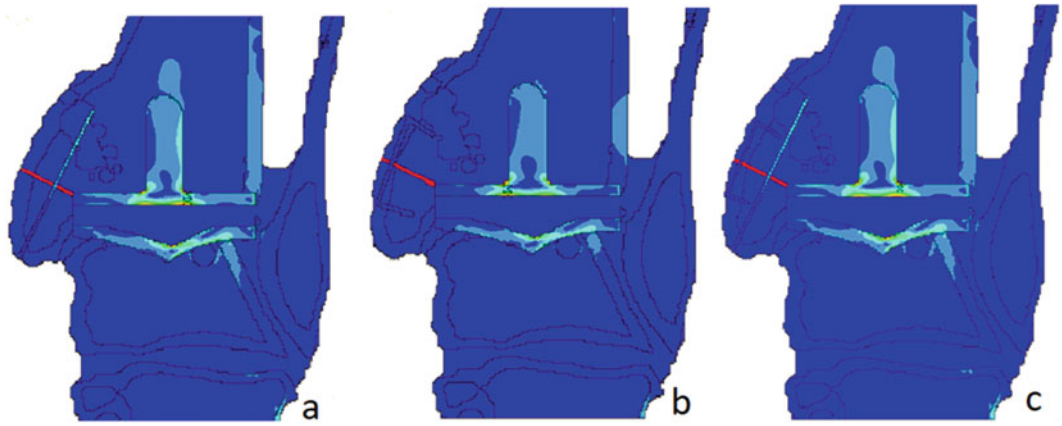

Fig. 2 Bone tension evaluation (stress distribution) arising at the bone-endoprosthesis area after total ankle arthroplasty and stabilization of the medial malleolus fracture. Fracture gap marked by a red stroke on the left-hand side: (**a**) cortical screw passing through the medial malleolus from its apex to the shaft of the tibia, transversely through the fracture gap; (**b**) Blount staple inserted with its tines above and below the fracture gap in the medial malleolus, stabilizing the fracture externally on the medial surface of the malleolus; (**c**) combination of the cortical screw and Blount staple

the medial malleolus, which is most frequently used in the clinic, a Blount staple, sometimes used in this type of fracture, and a combination of the two. The FEM model was based on the force load of $F_{\lambda} = 7.28$ N, determined from an average load of 1 N/1 mm^3.

3 Results

Maximum tensions locally found at the bone-implant interface in the *in silico* model was 8.4 MPa. This magnitude of tension was found without a fracture or healed fracture at the base of the medial malleolus. Similar tensions were noted in the malleolus fracture fixed with a screw alone, Blount staple, and a Blount staple and screw together. Tensions arose in the central parts of the tibial and talar components of the implant and the bone-implant interface. However, the area of maximum tension in the tibial component covered the entire cone base and was about three times larger than that in the talar component (Fig. 2). The greatest tension occurred along the central axis of the limb, and not in the fracture fissure or the material stabilizing the fracture. In each evaluated case, greater overloads were found in the cortical than medial layers of lateral tibia and talus walls.

When the fracture gap was stabilized with the Blount staple alone (Fig. 2b), tension along the cone of the tibial component was about 12% of the maximum tension. The use of a screw alone or together with the Blount staple (Fig. 2a, c) doubled up the tension on the medial side of the cone of the tibial component and about the immediate

vicinity. Additionally, stabilizing elements exert tension forces of similar magnitude in the extension of the tibia cone, targeting the posterior tibia wall. In the area of the medial malleolus, tensions were alike when stabilizing the fracture with a screw or together with the Blount staple. Tensions arising in the joining materials reached one-third of the maximum tension occurring in the entire biomechanical system in the case of screw alone or together with the Blount staple and one-fourth of the maximum tension in the case of Blount staple alone.

4 Discussion

In this study, we used a rapid FEM computer-driven modeling to evaluate tensions arising at the bone, endoprosthesis, and bone-endoprosthesis interface after total ankle arthroplasty. The modeling seems particularly useful in the assessment and prevention of the potential propensity for a fracture of the medial malleolus, an adverse event stemming from arthroplasty. The method is based on CT scans of the leg bones with an implanted ankle joint endoprosthesis, transformed into 2D images, aimed at providing the prompt evaluation results intraoperatively. The distribution of tensions arising after the endoprosthetic-related medial malleolus fracture was evaluated with the content of a stabilizing central cone in the tibial component. Noteworthy, we found differences in the magnitude and localization of tension depending on the way of fracture gap stabilization. In detail, the tension along the prosthetic cone of the tibial component approximated 12% of the maximum stress using the Blount staple. Stabilization with the Blount staple and screw together or a screw alone induced tensions on the medial side of the tibial cone component and its immediate vicinity twice as much.

According to the classification of Manegold et al. (2013), malleolus fractures are stratified into Type 1 – intraoperative fractures, type 2 – postoperative fractures resulting from a fall, and type 3 – fractures resulting from both bones overload and physiological stress on deficient elastic resistance. Concerning the exact location of a fracture site, the classification distinguishes type A – medial malleolus, type B – lateral malleolus, C – tibia, and D – talus. Fractures have the essential bearing on the implant stability, so an additional stratification distinguishes stable from unstable implants, anonymized as S and U, respectively, based on the X-ray or CT examination. Our present finite element model fits well into type 1AU intraoperative fractures. However, the model would also be adequate for those type 2 fractures in which there are no significant changes in bone anatomy apart from the fracture gap. Type 3 fractures are usually linked to altered bone structure. Therefore, the model would barely reflect clinical reality.

The prevalence of periprosthetic fractures arising during surgery can be as high as 20% (Brock et al. 2017; Manegold et al. 2013). In periprosthetic ankle fractures, it is recommended to use surgical stabilization to prevent displacement in the case of both intra- and postoperative fractures (Cody et al. 2018). The conservative treatment is recommended only for non-displaced fractures of the medial malleolus. Nonetheless, even in such fractures, the non-union occurs in 12–13% (Tsitsilonis et al. 2015). Intraoperative periprosthetic fractures are most often stabilized using Kirschner wires (K-wires), screws (Barnes et al. 2014), or locking plates (LCP) (Jiang et al. 2019). In some cases, Blount staples are used with satisfactory results (Schiedts et al. 1997). These methods have been studied in many aspects, but no consensus has been reached as to which one is the most effective. The prevalence of non-union in medial malleolus fractures ranges from 3% to 20% (Corey et al. 2019), which may result in implant destabilization. Jiang et al. (2019) have used the FEM for the evaluation of different types of stabilization in medial malleolus fractures and found that a screw inserted along the long axis of the medial malleolus from its apex, when the fracture gap runs horizontally, did not provide good results. Mounting a screw was not sufficient to prevent displacement. Further, tensions arising below the screw could chip the bone away and, as a result, the screw may go loose or fall out, causing severe pain as the screw head is close under the skin. The use of headless compression screws mitigates this

problem in the stabilization of medial malleolus fractures and reduces the percentage of non-unions. The perception of pain after endoprosthesis stabilization exceeds *in silico* analysis. The suboptimal nature of prosthetic stabilization using screws alone is consistent with our present results.

The locking plates are another way to stabilize malleolar fractures, which was subject to finite element analysis (Anwar et al. 2017). The plates prevent bone damage and the fallout of a screw by transferring a portion of the tension from the screw placed along with the locking plate to the shaft of the tibia. Unfortunately, there is a danger of breaking a locking plate. Besides, mounting the plates using screws is technically difficult and not always feasible due to a restricted intraoperative area. The current stabilization systems of prosthetic fractures of the medial malleolus still fall short of the requirements of effectiveness. The search continues for new methods that would provide better effectiveness and safety of the obtained fracture unions.

Blount staples have been used for years in epiphysiodesis, i.e., correcting discrepancies in limb length in children or for correcting valgus knees (genu valgum) or claw knees (genu varum), showing as good results as other corrective modalities, e.g., the eight-plate method (Rodrigues et al. 2020). In the treatment of medial malleolus fractures, the use of Blount staples is not common, but a study by Schiedts et al. (1997) has shown promising results. A new technique of Blount staples placement developed by Burghardt et al. (2012) requires a smaller skin incision and provides easier access to the bone and a stable union of the fracture, which raises interest in using it in a greater number of orthopedic procedures in both children and adults. Patients whose fractures are a consequence of the implantation of an ankle joint endoprosthesis may benefit from this method as well. Therefore, in the present study, we used the finite element method to model the stabilization of the medial malleolus fracture after ankle arthroplasty using Blount staples. We demonstrate a lower level of tensions developing in the endoprosthesis area, which carries a lower risk of endoprosthesis destabilization and procedure failure, and therefore a lower risk of reoperation and arthrodesis. This study has limitations inherent for the theoretical analysis that requires assumptions about the patient's condition, bone quality, and the type of fracture we are dealing with. Nonetheless, we believe we have shown that, contrary to the hitherto clinical routine of using screws, fracture fixation using the Blount staple leads to a lower bone tension about the stabilization of the medial malleolus fracture after ankle joint endoprosthesis. The assessment of a potential practical reference of the proposed fixation method as well as perioperative finite element modeling requires alternative clinically oriented study designs.

Conflicts of Interest The authors declare no competing interests in relation to this article.

Ethical Approval All procedures performed in this study involving human medical data complied with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This article concerned computer modeling based on anonymous clinical data. As it contains no identifiable personal data, no patient consent was required.

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Effects of Physical Activity at High Altitude on Hormonal Profiles in Foreign Trekkers and Indigenous Nepalese Porters

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Abstract

Altitude exposure affects hormonal homeostasis, but the adaptation of different populations is still not finely defined. This study aims to compare the mid-term effects of combining physical activity and altitude hypoxia on hormonal profiles in foreign trekkers coming from Italy versus indigenous Nepalese porters during a Himalayan trek. Participants (6 Italians and 6 Nepalese) completed a 300 km distance in 19 days of an accumulated altitude difference of 16,000 m, with an average daily walk of 6 h. The effect of high altitude on hormonal pathways was assessed by collecting blood samples the day before the expedition and the day after its completion. Foreign trekkers had an additional follow-up sample collected after 10 days. The findings revealed a different

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adaptation of thyroidal and gonadal axes to mid-term strenuous physical activity combined with high-altitude hypobaric hypoxia. The thyroid function shifted to the protective mechanism of low free triiodothyronine (FT3), whereas the gonadal axis was suppressed. The Italian trekkers and Nepalese porters had lower total testosterone and 17-β-estradiol levels after the expedition. At the follow-up, the Italians had increased testosterone values. Prolactin secretion decreased in the Italians but increased in the Nepalese. We conclude that exposure to high-altitude affects the hormonal axes. The effect seems notably pronounced for the hypothalamus-pituitary gonadal axis, suppressed after high-altitude exposure.

Keywords

Foreign trekkers · High altitude · Hormonal axis · Hypobaric hypoxia · Nepalese porters · Physical activity

1 Introduction

From the pioneering work of Hans Selye on endocrinology and adaptation syndrome, the hypothalamic-pituitary axis has been recognized as the main player in the homeostatic adaptation to psychological or physical stressors (Rochette and Vergely 2017). Among the environmental stressors, high altitude has been steadily growing in interest during the last decades, considering the spread of altitude traveling. High altitude affects human systems impacting their physiological activities due to hypobaric hypoxia and climatic condition. It has been shown that central and peripheral endocrine functions are affected by high altitude, usually above 5000 m, barring persistent human colonization (von Wolff et al. 2018). The high-altitude hypoxic model is usually adopted to study the pathophysiologic effects of low oxygen tension in the aging process or cardiorespiratory disorders (Verratti et al. 2016; Cataldi and Di Giulio, 2009). Chronic conditions can also impair hormonal metabolism affecting target organs' activities. However, findings in various studies are highly contentious, which may be caused by the wide diversity in the length and degree of hypoxic exposure, gender, age, and environmental conditions (Keenan et al. 2019; Verratti et al. 2017; Park et al. 2014).

Humans of different ethnicity, exposed from birth to different levels of hypoxia, respond and adapt to acute and chronic high-altitude exposure in diverse ways (Magliulo et al. 2020; Verratti et al. 2021). Comparisons of changes in hormonal profiles between different ethnicities, including sojourners and natives, in relation to high altitude are scarce. Researchers have focused on highland dwellers, trekkers, or climbers, somehow leaving out a population of altitude porters who have a specific geographic origin and often suffer from high altitude illness (Dawadi et al. 2020). The motivation behind the study of porters also lies in their legendary performance during uphill loaded locomotion, playing a fundamental role in the success of altitude expeditions (Minetti et al. 2006), as opposed to altitude travelers for whom the physical strain involved limits the performance and poses substantial stress. In this study, we address the effects of altitude hypoxia on hormonal profiles in foreign trekkers versus indigenous Nepalese porters during an altitude trek in the Himalayas.

2 Methods

2.1 Participants and Study Protocol

This study was conducted in accord with the STAR Data Reporting Guidelines for Clinical High Altitude Research (Brodmann Maeder et al. 2018). Six Italian trekkers and six indigenous native Nepalese porters took part in the trial, which was a separate ramification of the research project "Kanchenjunga Exploration & Physiology". The basic demographics of the participants are presented in Table 1. Participants completed a 300 km distance in 19 days of an accumulated altitude difference of 16,000 m, with an average daily walk of 6 h. The route involved demanding ascents and descents in the Kanchenjunga region in the Himalayas in Nepal (Fig. 1). The

Table 1 Participants' characteristics – The Himalayan "Kanchenjunga Exploration & Physiology" expedition

Ethnicity	Gender	Age (year)	BMI pre (kg/m^2)	BMI post (kg/m^2)
Caucasian (Italians)	Female	36	25.07	24.65
	Male	63	28.91	27.34
	Male	59	21.91	21.35
	Male	25	24.31	23.13
	Male	32	24.14	23.14
	Male	48	30.54	27.98
Mean ± SD		44 ± 15	25.8 ± 3.3	24.6 ± 2.6
Indo-Aryan (Nepalese)	Male	26	26.49	25.66
	Male	18	17.51	16.88
	Male	39	22.99	22.38
	Male	40	28.83	27.77
	Male	30	29.41	28.38
	Male	29	20.94	21.34
Mean ± SD		30 ± 8	24.4 ± 4.7	23.7 ± 4.4

BMI Body mass index, a day before and a day after the expedition

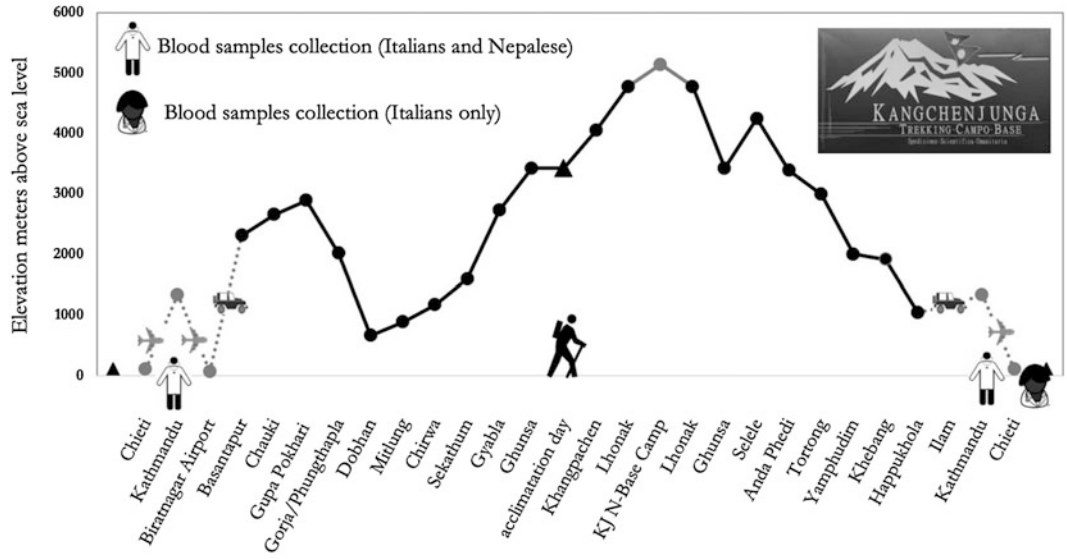

Fig. 1 Altimetric plan of "Kanchenjunga Exploration & Physiology" expedition

expedition was supervised by a trained doctor who monitored symptoms, peripheral blood oxygen saturation (SpO_2), arterial blood pressure, and anthropometric data throughout the whole period. None of the participants suffered acute mountain sickness.

Blood samples were collected from the antecubital vein, stored in vacutainer serum tubes, and immediately centrifuged at 3000 rpm × 10 min in the Bir Hospital (Kathmandu, Nepal, 1340 m), the day before and the day after the Himalayan trek. The serum was stored frozen at −5 °C and transported to Italy for later analyses in the Laboratory of Clinical Pathology of Teramo Hospital in the city of Teramo. Additionally, Italian participants' serum was collected and analyzed at a 10-day follow-up once they returned to Italy.

The blood content of hormones was determined using the immuno-chemiluminescence

assay in the ADVIA Centaur XP Immunoassay System (Siemens Healthcare; Erlangen Germany). We assayed thyroid function – free triiodothyronine (FT3), free thyroxine (FT4), thyroid-stimulating hormone (TSH), the hypothalamus-pituitary-gonadal axis – total testosterone, follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol, and the prolactin cortisol pathways.

2.2 Statistical Analysis

Normality of data distribution was assessed with the Shapiro-Wilk, the equality of variances with the Levene test, and whether the two data sets came from populations with a common distribution was determined with the quantile-quantile (Q-Q) plots. Considering the presence of missing points, we run a mixed-model analysis for all the parameters (Armstrong 2017). Specifically, we used the general linear mixed model (GLMM), with restricted maximum likelihood (REML) estimation and likelihood ratio testing (LRT) for random effects, time × ethnicity comparisons, and participants as the random variable. In the case of foreign participants, this analysis concerned pre vs. post vs. follow-up comparisons. The post hoc tests were conducted with the Bonferroni correction for multiple comparisons. The Satterthwaite method for degrees of freedom was used, and partial eta squared (η^2_p) and partial omega squared (ω^2_p) were calculated as measures of effect size (Fritz et al. 2012). The analysis was performed using R-based open-source Jamovi v1.2.5.0 software (https://www.jamovi.org).

3 Results

3.1 Thyroid Function

Thyroid-Stimulating Hormone Changes in TSH in both Italian and Nepalese participants after altitude exposure were unremarkable (Table 2).

Free Triiodothyronine (FT3) The investigation of thyroid function showed a reduction in FT3 concentration from pre-to-post altitude exposure ($p = 0.012$, $\eta^2_p = 0.525$, $\omega^2_p = 0.448$). The time × ethnicity comparison revealed a tendency for a

Table 2 Hormonal blood serum content in the Italian trekkers and Nepalese porters during exposure to high altitude in Kanchenjunga Exploration & Physiology" expedition

	Pre	Post	Follow-up
	TSH (μg/mL)		
Italians	1.26 ± 0.66	1.20 ± 0.64	1.08 ± 0.73
Nepalese	1.73 ± 0.81	1.61 ± 0.58	–
	FSH (mUI/mL)		
Italians	5.11 ± 1.48	4.96 ± 1.00	5.07 ± 0.96
Nepalese	5.84 ± 2.00	5.05 ± 2.36	–
	LH (mUI/mL)		
Italians	2.73 ± 1.00	3.26 ± 1.05	3.33 ± 0.55
Nepalese	4.26 ± 2.42	4.60 ± 1.61	–
	Cortisol (μ g/mL)		
Italians	9.41 ± 2.52	10.30 ± 2.73	10.81 ± 3.78
Nepalese	9.59 ± 3.79	8.59 ± 2.41	–

TSH Thyroid-stimulating hormone, *FSH* Follicle-stimulating hormone, *LH* Luteinizing hormone. Measurements were done a day before (pre) and a day after (post) the trek in the blood serum. An additional measurement was done at a 10-day follow-up in the Italians only. Changes in the serum content of hormones depicted in the table both between pre/post altitude exposure and between the ethnicities failed to reach statistical significance

difference ($p = 0.078$, $\eta^2_p = 0.306$, $\omega^2_p = 0.212$), with the Italian participants showing a greater reduction (from 2.75 ± 0.15 to 2.20 ± 0.46 pg/mL; $p = 0.016$) than the Nepalese (from 2.76 ± 0.31 to 2.63 ± 0.23 pg/mL) in post hoc analysis. At a 10-day follow up, FT3 reverted to the baseline level in the Italians (2.87 ± 0.35 pg/mL), as revealed by the pre vs. post vs. follow-up comparison ($p = 0.011$, $\eta^2_p = 0.703$, $\omega^2_p = 0.599$), and significant post hoc differences were found (pre vs. post: $p = 0.037$ and post vs. follow-up: $p = 0.020$). The random effect of participants was not significant, and the effect sizes for the overall model were as follows: R^2 marginal = 0.352, R^2 conditional = 0.569.

Free Thyroxine (FT4) In the Italian participants, FT4 content increased from pre-to-post altitude exposure, the increase persisted at a 10-day follow-up (1.07 ± 0.20 pg/mL, 1.21 ± 0.08 pg/mL, and 1.20 ± 0.09 pg/mL, respectively). In the Nepalese, we found an opposite trend, with FT4 reduction after altitude exposure (1.27 ± 0.14 pg/mL and 1.15 ± 0.08 pg/mL, respectively). Moreover, time × ethnicity comparison revealed a significant difference ($p = 0.025$, $\eta^2_p = 0.443$, $\omega^2_p = 0.359$). The random effect of participants was not significant, and the effect sizes for the overall model were as follows: R^2 marginal = 0.248, R^2 conditional = 0.425.

3.2 Hypothalamus-Pituitary-Gonadal and Hypothalamus-Pituitary-Adrenal Axes

Gonadotropins Changes in FSH and LH in both Italian and Nepalese participants after altitude exposure were unremarkable (Table 2).

Testosterone The content of total testosterone decreased from pre-to-post altitude exposure, albeit the changes were of borderline significance ($p = 0.109$, $\eta^2_p = 0.260$, $\omega^2_p = 0.164$), with the Nepalese participants having a greater reduction (from 4.56 ± 0.82 ng/mL to 3.40 ± 1.30 ng/mL) when compared with the Italians (from 4.86 ± 1.68 ng/mL to 4.35 ± 0.95 ng/mL); the difference between the two ethnic groups was insignificant (Fig. 2). At a 10-day follow up in the Italian participants, the baseline testosterone significantly increased to 6.57 ± 1.38 ng/mL, as revealed by the pre vs. post vs. follow-up comparison ($p = 0.038$, $\eta^2_p = 0.675$, $\omega^2_p = 0.515$), and post-hoc differences were found (pre vs. post: $p = 0.161$ and post vs. follow-up: $p = 0.043$). The random effect of participants was not significant, and the effect sizes for the overall model were as follows: R^2 marginal = 0.181, R^2 conditional = 0.335.

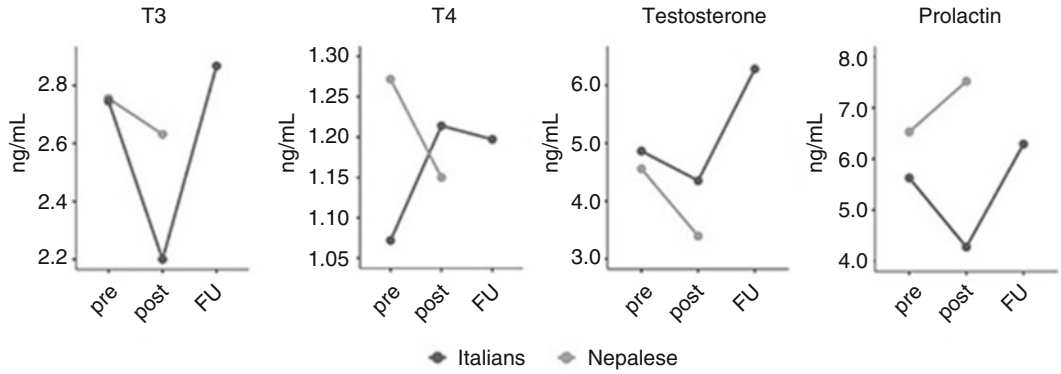

Fig. 2 Hormonal adaptations during exposure to high altitude in the Italian trekkers and Nepalese porters in Kanchenjunga Exploration & Physiology" expedition. Measurements were done a day before (pre) and a day after (post) the trek in the blood serum. An additional measurement was done at a 10-day follow-up (FU) in Italians only

17-β-Estradiol In the Italian participants, 17-β-estradiol content decreased from the pre-altitude exposure level of 30.98 ± 3.20 pg/mL to 11.80 pg/mL post-exposure, which was the lower detection limit, and reverted at a 10-day follow-up to 30.52 ± 2.73 pg/mL. In the Nepalese, a reduction in 17-β-estradiol after exposure was variably expressed; three of them had a massive decrease from 19.60 ± 3.36 pg/mL to below the detection limit of 11.80 pg/mL, another two had a modest decrease, on average, from 24.05 pg/mL to 21.68 pg/mL, and it tended to increase in one subject from 11.80 pg/mL to 12.80 pg/mL.

Stress Hormones Changes in cortisol in both Italian and Nepalese participants after altitude exposure were unremarkable (Table 2).

Considering prolactin, it decreased from pre-to-post altitude exposure in the Italian participants to bounce back, exceeding the baseline level at a 10-day follow-up (5.63 ± 1.39 ng/mL, 4.28 ± 1.34 ng/mL, and 6.11 ± 0.19 ng/mL, respectively), as revealed by the pre vs. post vs. follow-up comparison ($p = 0.009$, $\eta^2_p = 0.741$, $\omega^2_p = 0.642$), and post-hoc differences were found (pre vs. post: $p = 0.068$ and post vs. follow-up: $p = 0.010$). Contrarily, in the Nepalese, prolactin increased from pre-to-post exposure (6.53 ± 2.38 ng/mL and 7.52 ± 2.22 ng/mL, respectively); time × ethnicity comparison revealed a borderline difference ($p = 0.148$, $\eta^2_p = 0.218$, $\omega^2_p = 0.120$). The random effect of participants was not significant, and the effect sizes for the overall model were as follows: R^2 marginal = 0.281, R^2 conditional = 0.432.

4 Discussion

High altitude affects human systems influencing their physiological activities due to hypobaric hypoxia and climatic environment. Hypoxia per se influences oxidative metabolism, stimulating compensatory mechanisms to ensure proper oxygenation of tissues (Di Giulio et al. 2006). Central and peripheral endocrine functions, such as hormonal pathways, are affected by altitude above 5000 m, preventing persistent human colonization (von Wolff et al. 2018). In the present chapter, we investigated whether a combination of physical effort and hypobaric hypoxia was associated with a perturbation of hormonal profile in a group of foreign trekkers coming from Italy when compared with indigenous Nepalese porters.

The thyroidal axis showed an adaptive mechanism characterized by a reduction in the serum level of FT3 both in foreign and Nepalese trekkers. The FT4 level showed ethnically reversing features, increasing in the foreigners and decreasing in the Nepalese. The TSH level remained unchanged. The FT3 and FT4 levels reverted to the baseline pre-altitude exposure value in the foreign trekkers at a 10-day follow-up. Richalet et al. (2010) have reported a 16% increase in both total and free T3 after 3–4 days of stay at 4350 m. Contrarily, we found a reduction in T3 after the altitude trek, which was more accentuated in the foreign trekkers than the Nepalese porters. A reduction in T3 is typical of non-thyroidal illness syndrome (NTIS), which occurs in various stressful non-thyroidal illnesses and during starvation. Under such conditions, peripheral conversion of T4 to T3 is reduced in the presence of normal thyroid hormone secretion resulting in an FT3 decrease, while FT4 may be unchanged, decreased, or increased. The NTIS is underlain by impaired conversion of T4 to T3 due to enzymatic dysfunction of deiodinases. The low-T3 syndrome caused by reduced peripheral conversion of the prohormone T4 is also present in chronic inflammatory diseases. As a result, inflammation disrupts redox homeostasis, inhibiting the hypothalamic-pituitary-thyroid axis. Hypothyroidism that follows exacerbates the redox homeostasis disruption, further worsening the activity of deiodinase. Our findings suggest that a reduction in T3 might involve the same inflammatory redox pathway that drives the low-T3 syndrome (Mancini et al. 2016). Further, there is a biological plausibility that indigenous porters in Nepal have an innate protective mechanism sparing the function of deiodinases.

We found in this study that the gonadal hormone axis was partially suppressed as confirmed by a non-significant reduction in testosterone levels and a significant decrease in 17-β-estradiol after the expedition in both foreign and Nepalese participants. These findings are in line with those of Benso et al. (2007), who found reductions in serum testosterone in eight mountaineers at the base camp located at 5200 m during a Mount Everest expedition. Marinelli et al. (1994) have reported a prompt decrease in serum testosterone occurring within 24 h after exposure to high altitude. Thus, the possibility arises that an inappreciable reduction in testosterone we observed after days of trekking during the expedition could be due to a delayed blood sampling which enabled the rebound of testosterone level, likely triggered by increased LH. Hypobaric hypoxia, rather than physical effort, seems the main reason for a transient reduction in testosterone as prolonged strenuous physical activity in endurance athletes or marathon runners at sea level fails to show appreciable effects on circulating reproductive hormones (Lucía et al. 1996; Jensen et al. 1995; Bagatell and Bremner 1990). Noteworthy, we found a significant increase in serum testosterone in foreign trekkers at follow-up, the finding in line with a previous study in which testosterone increased 10 days after the end of prolonged trekking at 5900 m in seven male mountaineers, which could be related to changes in the body composition (Pelliccione et al. 2011).

We also found that serum prolactin decreased in the Italian trekkers at high altitude with a reversal to the baseline level at the 10-day follow-up. It is a reasonable assumption that altitude hypoxia mitigates the activation of lactotroph cells in the anterior pituitary resulting in less prolactin secretion, which may be a sign of general bodily suffering of foreign sojourners at altitude. Likewise, Benso et al. (2007) have found a significant increase in serum prolactin in Caucasian mountaineers after climbing Mount Everest. The authors suggest changes in prolactin reciprocate those in testosterone. Somewhat in line with this suggestion, we found that prolactin increased from the pre-to-post trek in the Nepalese porters, a reverse reflection of those in testosterone. The ingenious high-altitude settlers perform physically better compared to foreigners even though they usually are exposed to a greater physical load.

The present study also shows that serum cortisol was not appreciably affected by high-altitude trekking. The lack of effect of cortisol content is in line with the findings of Wolff et al. (2018), who have conducted a major study on cerebral, cardiovascular, pulmonary, and humoral adaptations to prolonged hypobaric hypoxia during an ascent to Mt. Himlung Himal (7126 m) in Nepal, involving forty healthy sojourners, 21 men and 19 women aged 18–70. In that study, serum cortisol was slightly modulated with increasing altitude, changes were unrelated to sex or arterial oxygen saturation, and the level of cortisol after the expedition was akin to that before it. That study, in line with our present findings, also shows that prolactin secretion and thyroid function are affected by increasing altitude and the gonadal hormonal axis is suppressed, with reversal of changes to the baseline levels at a follow-up.

In conclusion, we believe we have shown that brain-driven humoral responses are modulated by a prolonged strenuous effort during a Himalayan high-altitude trek. The accompanying hypobaric hypoxia appeared to notably affect the hypothalamus-pituitary gonadal axis suppressing serum testosterone and 17-β-estradiol contents; the effects were more pronounced in indigenous Nepalese porters than in foreign Caucasian sojourners. Hypobaric hypoxia also shifted the thyroid metabolic function to the protective way of low free triiodothyronine level, the effect was more pronounced in Caucasian sojourners. There is a degree of ethnic diversity in specific humoral changes toward the stronger adaptive downregulation of humoral function in high-altitude native Nepalese. This adaptation may underlie their superior performance in the physically loaded condition, when compared to foreign sojourners, due possibly to the ensuing lower cellular ATP demand mitigating the effects of

oxygen depletion at high altitude. Generally, however, the ethnic differences in humoral responses were modest and reversible on return from the trek in both Caucasians and native Nepalese. The exact molecular mechanisms of humoral changes underlying the effects of high-altitude hypobaric hypoxia could not be resolved in this observational study. Further research, particularly considering the diurnal response, in addition to sequential assessments during altitude exposure, is needed to provide insights into ethnicity-dependent differential responses to physical effort at high altitude.

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Competing Interests The authors declare no competing interests in relation to this chapter.

Ethical Approval All procedures performed in studies involving human participants were in accord with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This project was approved by the Ethics Review Board of the Nepal Health Research Council (NHRC).

Informed Consent Written informed consent was obtained from all individual participants included in the study.

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Influence of Parathyroidectomy on Sleep Quality in Primary Hyperparathyroidism

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Abstract

We investigated the impact of parathyroidectomy on sleep quality in patients with primary hyperparathyroidism (pHPT). Thirty consecutive patients with pHPT were enrolled in the study within 1 year. pHPT was diagnosed by typical symptoms accompanied by an elevated level of parathormone. The Pittsburgh Sleep Quality Index (PSQI) was used for the evaluation of sleep 1 day before and 6 months after parathyroidectomy. The mean total PSQI score was elevated to 6.8 ± 0.6 points before surgery, which was in the pathological cut-off of greater than ≥5, indicating impaired sleep quality. After parathyroidectomy, the total score declined insignificantly, amounting to 5.6 ± 0.4 ($p > 0.05$). Nevertheless, the number of patients with a score of ≥5 before surgery decreased from 21 (70%) to 16 (53%) after surgery. There also was a significant improvement in sleep latency ($p = 0.05$) and sleep efficiency ($p = 0.02$) domains of PSQI. We conclude that 70% of patients with untreated pHPT suffered from sleep disorders that improved after parathyroidectomy. The clinical consequence is that patients with pHPT should be questioned about having sleep disorders, which might influence the decision-making concerning parathyroidectomy. With the relation reversed, patients without pHPT but suffering from sleep disturbance should be tested for pHPT.

Keywords

Hyperparathyroidism · Parathyroidectomy · Sleep disorders · Sleep quality

1 Introduction

Primary hyperparathyroidism (pHPT) is caused by adenomas of the parathyroid gland. This benign disease is characterized by parathyroid hyperfunction. Serum parathormone and calcium levels are elevated. A definite therapy can only be achieved by parathyroidectomy

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(Miedlich et al. 2002). Classical symptoms and comorbidities of pHPT are kidney stones, bone diseases, and neuromuscular dysfunction. Patients also suffer from other unspecific symptoms like weakness, fatigue, depression, anxiety, irritability, cognitive dysfunction, and sleep disturbance (Liu et al. 2020; Pasieka et al. 2009; Mittendorf et al. 2007; Weber et al. 2007; Dotzenrath et al. 2006; Lundgren et al. 1998). This study aims to investigate the influence of parathyroidectomy on sleep quality in patients with pHPT.

2 Methods

2.1 Patients and Protocol

A total of 30 consecutive patients with pHPT were enrolled in the study for 1 year. There were 26 women and 4 men in the study, aged 57 ± 2 years, with the body mass index (BMI) of 26.3 ± 0.9 kg/m^2. The pHPT was diagnosed due to the presence of typical symptoms and the elevated serum levels of parathormone and calcium. All patients underwent a parathyroidectomy. The sleep quality was evaluated 1 day before and 6 months after surgery using the Pittsburgh Sleep Quality Index (PSQI). Exclusion criteria were a PSQI score of ≤3 according to the classification of the American Society of Anesthesiologists (ASA), secondary HPT, and the inability to understand the German language. There were no dropouts.

2.2 Pittsburgh Sleep Quality Index (PSQI)

The PSQI is a validated questionnaire that assesses subjective sleep quality during the last 4 weeks. The survey contains 18 items in 7 domains. Aside from general sleep quality, it assesses sleep latency, efficiency, duration, sleep-disturbing events, the use of sleep medication, daytime sleepiness, and fatigue (DGSM 2007a, b). The domains are scored from 0 to 2. These values sum up for the total score ranging from 0 to 21. The higher the total score, the worse is the sleep quality. When the total score is ≥5, the patient is assumed to suffer from sleep disturbance (Buysee et al. 1989).

Data were expressed as mean ± SD. Intergroup differences were tested using Student's *t*-test for independent samples or by a χ^2-test in case of non-normal distribution. A p-value <0.05 defined statistically significant differences. The analysis was done using MS Excel for Windows.

3 Results

3.1 Patients

The serum PTH and Ca^{2+} before parathyroidectomy were increased to 211.7 ± 135.3 ng/L and 2.8 ± 0.2 mmol/L, respectively. Six months after the operation, both declined to the normal range of 34.9 ± 14.4 ng/L and 2.3 ± 0.1 mmol/L, respectively. Patients had several typical pHPT comorbidities before parathyroidectomy, such as osteoporosis – 3 (10%) patients, a fracture of a lumbar vertebra – 1 (3%), kidney stones – 4 (12%), and gastrointestinal disorders – 7 (23%).

3.2 Pittsburgh Sleep Quality Index (PSQI) Score

The mean value of the total PSQI score was 6.8 ± 0.6 before parathyroidectomy. This score was in the pathological range of ≥5 in 21 out of the 30 (70%) patients, indicating impaired sleep quality. After surgery, the score tended to decline, amounting to 5.6 ± 0.4. It remained elevated above 5 points in 16 (53%) patients. The mean pre/postoperation difference of 1.2 ± 0.1 was

insignificant. The evaluation of specific PSQI domains yielded variable results.

Sleep Quality Before parathyroidectomy, 16 patients had "very good" or "quite good" subjective sleep quality and 14 patients "quite bad" or "very bad" sleep quality. After the operation, 20 patients had "very good" or "quite good" sleep quality and 10 patients had "quite bad" or "very bad" sleep quality. The tendency for the amelioration of sleep disturbance after surgery failed to reach statistical significance ($p = 0.29$).

Sleep Latency Before parathyroidectomy, 21 patients scored 0 or 1 (0 = sleep latency <30 min, 1 = sleep latency >30 min less than once in a week), 9 patients scored 2 or 3 (2 = sleep latency >30 min two or three times a week, 3 = sleep latency >30 min more than three times a week). After surgery, 27 patients scored 0 or 1 and 3 patients scored 2 or 3, which led to a significant reduction in sleep latency ($p = 0.05$).

Sleep Duration Before parathyroidectomy, 19 patients slept for six or more hours *per* night. After surgery, the number of such patients increased to 24. Although this difference failed to reach statistical significance, there was a consistent impression that parathyroidectomy tended to extend sleep duration.

Sleep Efficiency Sleep efficiency is defined as the percentage of time spent asleep while in bed (DGSM 2007a). A normal level of sleep efficiency is set at 85% or higher. Before parathyroidectomy, 11 patients had sleep efficiency greater than 85%. After surgery, 20 patients reached this level of sleep efficiency; the effect was significant ($p = 0.02$).

Sleep Disorders Concerning sleep disorders, patients had to answer the question of how often *per* week and for what reason they wake up at night. Before parathyroidectomy, 20 patients reported up to 2 awakenings *per* week, which was considered normal, and 10 patients had more than 2 awakenings *per* week. Surgery failed to appreciably change the number of nightly awakenings, with 19 and 11 patients, respectively ($p = 0.79$).

Sleep Medications Patients had to answer the question how often they used sleep medications in the past 4 weeks, prescribed or acquired over the counter. Before parathyroidectomy, 27 patients reported taking sleep medications less than once a week compared to 28 patients after the operation. The remaining patients were taking sleep medications once a week or more often. Thus, the surgery failed to influence the necessity to use sleep medications ($p = 0.64$).

Daytime Sleepiness The assessment of daytime sleepiness consisted of two questions. The first question concerned the potential problems with staying awake during car driving, eating, or social encounters and the other concerned the problems with handling daily chores in the past 4 weeks. Twenty-two patients before parathyroidectomy and 26 after it did not report the increased daytime sleepiness, as opposed to 8 and 4 patients, respectively, who did report an increase in sleepiness. There was no significant pre/postsurgery change in daytime sleepiness ($p = 0.20$). The PSQI assessment data above outlined are summarized in Fig. 1.

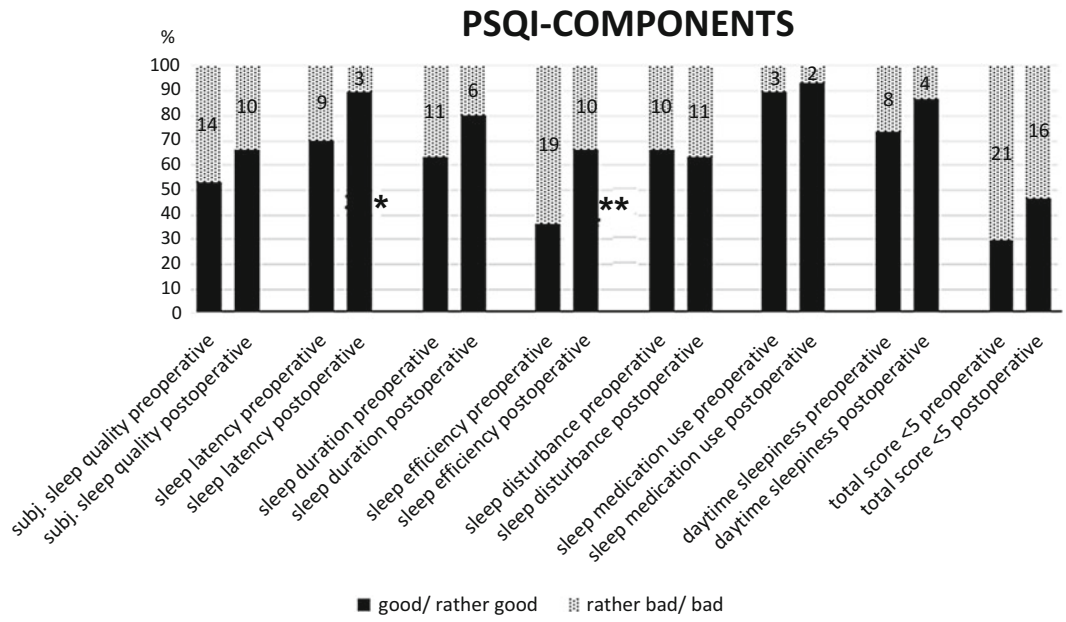

Fig. 1 Percentage and the absolute number of patients with primary hyperparathyroidism with good/rather good (black) versus bad/rather bad (dotted) scores in Pittsburgh Sleep Quality Index (PSQI) pre/post parathyroidectomy. Total number of patients in each vertical bar was 20; *p = 0.05; **p = 0.02

4 Discussion

Several studies of the last years have investigated cognitive and neuro-psychological changes, sleep quality, and the quality of life in patients with pHPT (Kearns et al. 2019; Trombetti et al. 2016; Wu and Yeh 2016; Alex et al. 2013; Morris et al. 2010; Adler et al. 2009; Perrier et al. 2009; Repplinger et al. 2009; Esposito et al. 2008; Tsukahara et al. 2008; Caillard et al. 2007; Perrier et al. 2006; Roman-et al. 2005; Quiros et al. 2003; Prager et al. 2002; Pasieka et al. 2002; Burney et al. 1998; Burney et al. 1996; McAllion and Paterson 1989; Numann et al. 1984). Joborn et al. (1988) were the first to set a focus on the importance of sleep disorders in patients with pHPT by the demonstration of a shortened sleep in such patients.

The pathophysiology of sleep disorders in pHPT is multifactorial. Possible reasons are in the first line all the symptoms connected with the disease, such as bone pain, myalgia, anxiety, and kidney stones. Another cause might be cardiac autonomic nerve dysfunction altering the circadian rhythm with an impact on sleep (Logue et al. 1990). Furthermore, the elevated PTH stimulates osteoblasts, which leads to an increase in interleukin-6 (IL-6) content that may reduce sleep efficiency. Elevated serum calcium reduces the regional cerebral blood flow by vasoconstriction, which may also cause sleep disorders. Autonomic dysfunction and biochemical changes characteristic of pHPT revert after parathyroidectomy when the PTH serum level normalizes (Murray et al. 2014; Mittendorf et al. 2007; Perrier et al. 2006; Safley et al. 2004; Mjäland et al. 2003; Nilsson et al. 2003).

Literature data on the impact of hyperparathyroidism on sleep quality and the effects of parathyroidectomy are scarce and contentious. Walker et al. (2004) and Joborn et al. (1989) have found insignificant changes in sleep quality after surgery. Contrarily, some other authors have found that sleep quality somehow improves after surgery (Friedman et al. 2005; Hong et al. 2005;

Opp 2005). Esposito et al. (2008) have reported an extension of patients' sleep time after surgery. These authors also report a high prevalence of sleep disorders in pHPT before surgery, reaching 95.5% of patients. We have previously demonstrated significant amelioration of cognitive functions in pHPT patients after parathyroidectomy (Dotzenrath et al. 2006), the finding recently confirmed by Shah-Becker et al. (2017). The present study expands on those findings by investigating the prevalence of sleep disorders in pHPT and the effects of parathyroidectomy on sleep quality. Using the PSQI questionnaire, we found a 70% prevalence of sleep disorders in pHPT patients before surgery. This figure is higher than the 25–30% estimated in the general population (DGSM 2015; DGSM 2009). Six months after parathyroidectomy, the prevalence was reduced to 54%. Statistically, the reduction was not significant, with the prevalence of sleep disorders remaining elevated when compared to the general population. However, we found that two specific sleep quality components, sleep latency and efficiency, significantly improved after parathyroidectomy. The score of the former decreased and the latter increased. The improvements are in line with a better quality of sleep after surgery.

In the present study, we did not notice changes in the other PSQI items such as sleep duration, sleep-disturbing events, the use of sleep medication, and daytime sleepiness and fatigue. The lack of changes in sleep duration is in line with the findings of Walker et al. (2004) and Joborn et al. (1989) but contrasts the elongation of sleep duration observed by Esposito et al. (2008). There is, however, a notion that sleep duration may not be a good reflection of impaired sleep quality (DGSM 2009). Sleep latency and efficiency are the essential features of sleep. Neither do patients with pHPT commonly suffer from excessive daytime sleepiness, characteristic of sleep-disordered breathing (Pasieka et al. 2009).

The clinical consequence is that patients with pHPT should be systematically questioned about the presence of sleep disorders, especially insomnia rather than hypersomnia. The presence of insomnia-impaired sleep might be an additional indication for performing parathyroidectomy. Vice versa, it should be considered to test patients without pHPT but suffering from insomnia for the presence of pHPT.

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Conflicts of Interest The authors declare no conflicts of interest concerning this chapter.

Ethical Approval All procedures performed in studies involving human participants were in accord with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study was approved by the Ethics Committee of the Medical Faculty of the University of Witten/Herdecke, Germany.

Informed Consent Written informed consent was obtained from all individual participants included in the study.

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Mini Craniotomy in the Management of Supratentorial Spontaneous Intracranial Hemorrhage: A Single-Center Outcome of the Minimally Invasive Treatment

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Abstract

Hemorrhagic stroke accounts for a significant proportion of mortality and confers a poor quality of life with high dependency among survivors. Surgical evacuation of hematoma has the advantage of rapidly controlling the increased intracranial pressure, halting the ongoing herniation syndrome, and mitigating the secondary cascades of events mediated by the inflammatory and blood degradation products. The advantage is hindered by the concurrent insult to the healthy brain tissue while passing through the normal brain tissue. Therefore, minimally invasive approaches to evacuate the hematoma are employed, but the need for an expensive surgical armamentarium and the expert multidisciplinary team is the bottleneck for their application, particularly in low-income nations. We herein performed a study upon the role of mini craniotomy open surgical method of evacuating hematoma in selected patients with supratentorial intracerebral hemorrhage. We found a significant reduction in the surgery length, minimized risk of post-surgery complications, shortened intensive care unit stay, and reduced mortality compared to the full-fledged craniotomy and endoscopy-guided surgery. There is a need for a large-scale randomized multicenter prospective study to verify the advantages of minimally invasive approaches in the management of symptomatic supratentorial intracerebral hemorrhages.

Keywords

Brain · Craniotomy · Hematoma · Intracerebral hemorrhage · Minimally invasive surgery · Mortality

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1 Introduction

Spontaneous intracerebral hemorrhage is responsible for 9–27% of all strokes (Feigin et al. 2009). The mortality rate within 30 days has been reported to be as high as about 30% (Smajlović et al. 2008). Long-term survivors are often saddled with permanent deficits, with up to 75% suffering a significant disability. Surgical intervention is recommended for patients with a clot volume from 20 mL to 80 mL, worsening neurological status in relatively young patients, and hemorrhage causing midline brain shift or raised intracranial pressure (Siddique and Mendelow 2000). Various surgical strategies have been described ranging from craniotomies, decompressive surgery, to minimally invasive modalities like a stereotactic or endoscopic-aided aspiration. Craniotomy or decompressive large craniectomy facilitates good evacuation of hematoma and ensures effective hemostasis. However, the morbidity associated with general anesthesia, prolonged surgery duration, and blood loss is the inherent limitations. In minimally invasive procedures, the morbidity of extensive craniotomy can be obviated. Conventional craniotomy has a high mortality rate of 22–36%. Minimally invasive procedures have a high evacuation rate, low incidence of complication, better protection of normal neural tissue, and fewer surgery-related injuries. Direct visualization of bleeding points and coagulation of the responsible vessels also is feasible. The incidence of infectious complications is low, skin incisions are smaller, and surgery and intensive care unit stay are shorter (Zhang et al. 2014). Minimal invasiveness also encompasses collateral economic benefits, which plays an essential role in the health sector of low-to-middle income nations. The endoscopy-assisted evacuation of intracerebral hematoma is associated with a minimal rebleeding, reaching a meager 0–3.3% when compared to the 5–10% of the classical craniotomy approach (Gaab 2011). Minimally invasive procedures, however, require sophisticated and expensive armamentariums and the expert staff team for conducting specialized procedures such as endoscopy with neuro-navigation and catheter drainage. This study presents clinical outcomes in patients with spontaneous intracerebral hemorrhage treated with mini craniotomy in a tertiary hospital in Nepal. Minimally invasive surgery was performed with rather limited resources based on standard neurosurgical instruments, optimized direct visualization, and was aided with controlled brain retraction.

2 Methods

2.1 Patients

Eighty-five stroke patients, aged 52 $\pm$ 9 (M/F – 69/16), with lobar cortical and basal ganglia hemorrhages, treated with the minimally invasive approach of mini craniotomy in a tertiary hospital in Nepal were included in the study. A volume of hematoma was calculated from computer tomography (CT) images according to Kothari et al.'s (1996) formula. The specific anatomical site of the basal ganglionic hemorrhage was set according to Chung et al.'s (2000) localization scheme.

Inclusion criteria were as follows:

- Patient's age above 15
- CT scan showing spontaneous lobar or basal ganglia hemorrhage.
- Hematoma volume $\geq$ 30 mL
- Glasgow Coma Scale (GCS) motor score on admission $\geq$4 points

Exclusion criteria were as follows:

- Hemorrhage caused by secondary factors like vascular malformations, tumors, or head injury
- Features of advanced herniation with infarction seen in the neuroradiologic examination
- GCS motor score on admission <4 points
- Multiple intracranial hemorrhages
- Clotting disorders
- Preexisting neurological deficits like previous intracerebral hematoma or infarct
- Gross ventricular enlargement
- Consent refusal

2.2 Sample Size Calculation

The minimum statistically meaningful sample size of patients for this study was calculated using the formula:

$$N = z^2 \, x \, p \, x \, q / d^2$$

where $Z = 1.96$ at 95% confidence interval

p = prevalence of hemorrhagic stroke taken as 30%
q = 1-p
d = margin of error taken as 10%

The result of the calculation was 80.8, and we included 85 patients in the study.

Overall, we recorded and evaluated the following elements: clinical and radiological outcomes, demographic data, hematoma volume, GCS on admission, duration of the surgical procedure, intraoperative and postoperative complications, and duration of stay in the intensive care unit (ICU) after surgery.

2.3 Surgical Approach

Surgery was performed under endotracheal general anesthesia. A linear skin incision was made. Mini craniotomy of 2.0–2.5 cm in diameter over a frontal or skull parietal area was depending on the shortest way to the epicenter of a hematoma. A cruciate dural opening followed by a small corticotomy was made over the relatively non-eloquent area. The hematoma was then evacuated using controlled suction aided with biopsy forceps to mince the hematoma. Hemostasis was achieved by continuous saline irrigation, pressure packing, using a bipolar cautery, and standard micro-neurosurgical techniques. The hematoma cavity was lined with fibrin sealant and the dura was left open and covered with gel foam. The bone was repositioned. The scalp was then closed in layers.

3 Results

Clinical features of hemorrhagic stroke patients are shown in Table 1. Although a documented history of arterial hypertension was present in 71 (83.5%) patients, compliance with antihypertensive treatment, the therapy was seen in only about one-third of them. The proportion of basal ganglia and cortical lobar bleeds was 3 to 1. In basal ganglia, the anatomically lateral variant was the most common hemorrhage site in 38 (59.4%) out of the 64 patients, followed by the massive hemorrhage in 19 out of the 64 (29.7%) patients, and the anterior variant in 7 (10.9%) out of the 64 patients. The mean volume of hematoma was 46.5 ± 10.5(SD) mL, the mean duration of the surgical procedure was 36.5 min, and the mean post-surgery time spent in the ICU was 3.2 ± 0.8 days. A difficulty in achieving hemostasis deep inside the hematoma cavity was seen in 2 cases only and the rebleeding occurred in 3 (3.8%) out of the 85 patients with re-evacuation carried out in 2 of them. Three (3.8) patients died, with pulmonary embolism accounting for two deaths and ventriculitis for one.

The mini craniotomy bone flap and the postoperative CT-reconstructed 3D skull image are schematically illustrated in Fig. 1. Representative examples of preoperative and postoperative CT images of the cortical and basal ganglia hemorrhage sites are demonstrated in Fig. 2.

4 Discussion

Primary injury caused by spontaneous intracerebral hemorrhage is mainly due to the mechanical mass effect of a hematoma. Secondary injuries are triggered by the developing inflammation and degraded blood products. The prime benefit of surgical evacuation is the rapid control of increased intracranial pressure, which mitigates the harmful cascade of cerebral herniation,

Table 1 Clinical features

Age (years)	52 ± 9
Male/female ratio	69/16; 4.3:1.0
History of hypertension (n)	71 (83.5%)
Compliance with antihypertensive medications (n)	30 (35.3%)
Mean volume of hematoma (mL)	46.5 ± 10.5
Basal ganglia/cortical bleeds ratio	64/21; 3:1
Anatomic distribution of basal ganglia bleeds	Anterior −7/64 (10.9%) Lateral – 38/64 (59.4%) Massive – 19/64 (29.7%)
Duration of surgical procedure (min)	36.5 ± 5.2
Complications	Rebleeding -3/85 (3.8%) Mortality - 3/85 (3.8%)
Duration of stay in the intensive care unit (days)	3.2 ± 0.8

Mean data are ±SD

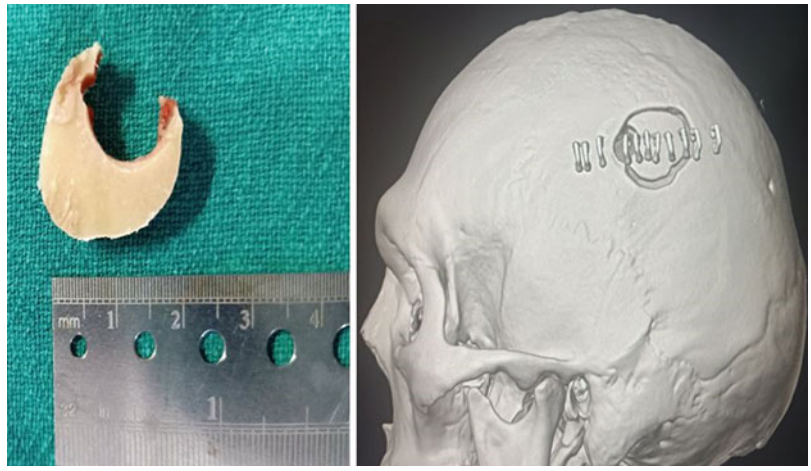

Fig. 1 A mini craniotomy bone flap and the post-operative computer tomography reconstructed skull image

restores cerebral perfusion, and inhibits the development of inflammation (Gaab 2011). The recent progress in minimally invasive surgical techniques to treat intracerebral hematoma, like endoscopy-guided stereotactic aspiration, is advantageous in that the inescapable trauma to the normal brain tissue is minimized and the clot hematoma is rapidly evacuated. A small surgical wound and limited intraoperative blood loss make wound healing better. Further, shortened surgery duration in relatively stable patients promotes early extubation, decreases the odds of postoperative pulmonary complications, and reduces the duration of patient stay in the ICU, all of which is conducive to faster patient recuperation and post-surgery rehabilitation.

Nonetheless, the control of bleeding and proper manipulation of instruments in the restrained operational space are limiting factors in minimally invasive surgical procedures that require much training and expertise (Ratre et al. 2018). The cost factor associated with the use of such methods also plays a role in low-to-middle income nations. The financial burden accounts for a significant proportion of "leave against medical advice", i.e., abandoning useful medical procedures (Hasan et al. 2019). An incomplete clot evacuation is another major concern. Classical craniotomy has an obvious advantage of decreasing the raised intracranial pressure along with the evacuation of a hematoma. The minimal approaches lag on this account, and there is a risk

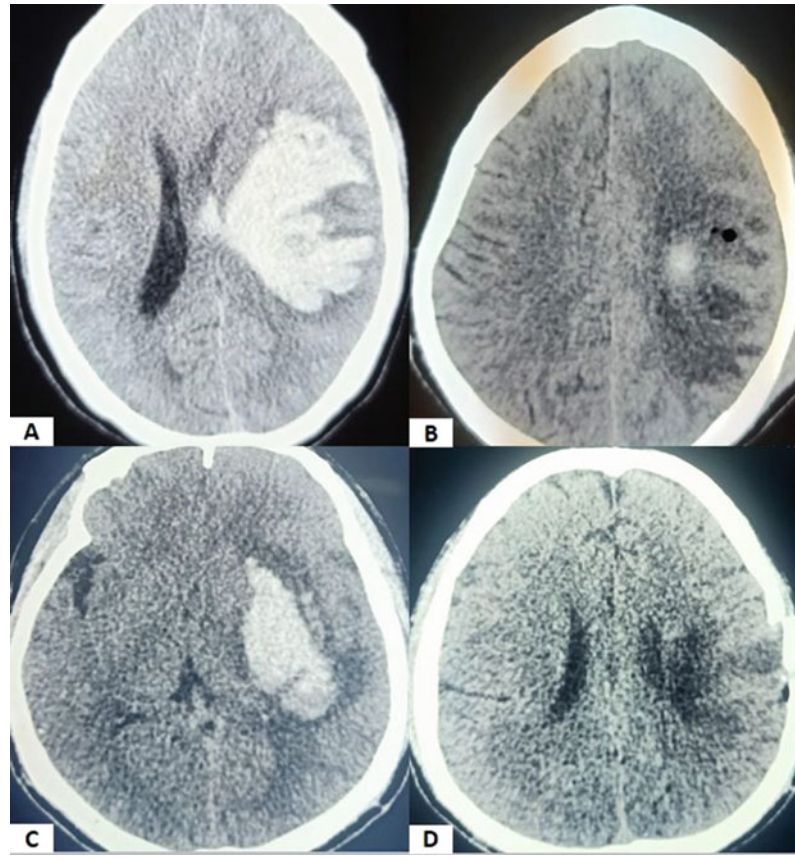

Fig. 2 Representative examples of preoperative and postoperative computer tomography images of cortical (panel A and B) and basal ganglia (panel C and D) hemorrhage sites

of brain edema in a subset of patients despite hematoma evacuation, necessitating the need for conversion to a larger craniotomy. A minimally invasive technique would not be recommended in patients with a low Glasgow Coma Scale score, moribund neurological status, and clinico-radiological features of advanced brain herniation. The minimal approach sometimes has a limitation of ensuring hemostasis, especially at the depth of a cavity, or complete hematoma removal concurrent with the minimum brain retraction. The technical difficulty of appropriate surgical light focusing at the depth of a cavity also is a major concern. In the present study, we thoroughly assessed the practical feasibility of performing the minimally invasive procedure for intracranial hematoma evacuation in a selected group of stroke patients. We herein report that the procedure can be safely undertaken with the application of basic neurosurgical instruments, fulfilling the required criteria of surgical treatment of the ailment, with add-on advantages of less surgical insult to the brain, less anesthesia, and perioperative patient stress, and faster recuperation.

A higher proportion of male patients were found to suffer from spontaneous intracranial hemorrhage in the present study, which is in line with some of the previous reports (Khallaf and Abdelrahman 2019). However, the gender predominance of brain hematoma is a contentious issue as some other reports point to a similar incidence rate in both sexes (van Asch et al. 2010; Appelros et al. 2009). The most common location of a spontaneous brain bleed among both conservatively and surgically managed patients is the basal ganglia area (Rychen et al. 2020). We confirmed the predominance of basal ganglia in the present study with 75.3% of patients having a hemorrhagic stroke in this area. Likewise, the

mean hematoma volume of 46 mL we found is in line with other reports (Luan et al. 2019; Rutkowski et al. 2019).

A previous comparative study showed a minimally invasive puncture and drainage as the least traumatic procedure with the least blood loss, followed by the endoscopy and craniotomy groups. However, the hematoma evacuation rate was lower in the minimally invasive puncture group (35.2%) compared with the other two groups where it amounted to 90.8% and 87.3%, respectively. Yet the minimally invasive puncture enjoyed the shortest treatment time of about 38 min, which is far less compared to the 98–120 min of the endoscopy-assisted removal of hematoma, with the latter being one-fourth the time taken for the classical craniotomy approach. On the downside, the minimally invasive puncture had the highest rebleeding rate. By contrast, a craniotomy was more effective in removing hematoma but resulted in marked trauma to the brain and also has the highest incidence of post-surgery pulmonary infection (Fu et al. 2019).

In the present study, the rebleeding occurred in just 3 (3.8%) out of the 85 patients. There also were three (3.8%) deaths. For comparison, there were 6 deaths out of the 40 (15%) patients and 4 out of the 25 (16%) patients undergoing minimally invasive craniotomy reported in the studies of Luan et al. (2019) and Rutkowski et al. (2019), respectively. Concerning other studies and other treatment modalities, data on the mortality rate are divergent. A study by Fu et al. (2019) has reported a mortality rate of 1.6% in the endoscopy-assisted management of intracranial hemorrhage, 3.6% in the minimally invasive puncture, and 5.0% in the craniotomy groups. By contrast, much higher mortality rates have been reported by Ratre et al. (2018) amounting to 11% in patients undergoing the endoscopy-assisted management of spontaneous bleed into the basal ganglia area and by Rychen et al. (2020) amounting to 21.3% in craniotomy with hematoma evacuation.

Pulmonary post-surgery embolism is a rare but mortal event. In this study, we noticed two such fatalities compared to one case in a study by Luan et al. (2019). We also noticed two (2.3%) cases of inflammation of the ependymal lining, with one of the ventriculitis being fatal. Surgical site infections are another leading cause of mortality among stroke survivors (Kuohn et al. 2020).

In conclusion, mini craniotomy evacuation of hematoma caused by spontaneous intracranial hemorrhage in patients suitable for this kind of surgery showed good clinical outcomes, minimizing the length of a surgical procedure, intraoperative and postoperative complications, length of stay in the ICU, and mortality rate when compared to reports on other treatment modalities such as classical craniotomy or endoscopy-assisted hematoma evacuation. The minimized surgical site infection and a shorter stay in the intensive care unit as seen in our patients promotes the "patient care bundle" approach by facilitating early rehabilitative programs. The relative ease of performing the procedure with basic neurosurgical instruments calls for the appraisal and validation of these findings in randomized multicenter prospective studies.

Conflicts of Interest The authors declare no conflicts of interest concerning this article.

Ethical Approval All procedures performed in studies involving human participants were in accord with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study was approved by the Institutional Review Committee of the College of Medical Sciences in Chitwan, Nepal; permit no. 2020-107.

Informed Consent Written informed consent was obtained from all individual participants included in the study or their guardians.

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Correction to: Outcome of Repeat Pulmonary Metastasectomy

Marcus Krüger, Katharina Franzke, Taufiek Konrad Rajab, Fabian Nadler, Moritz Möbius-Winkler, Norman Zinne, Daniel Schulz, Miriam Möller, Wolfgang Schütte, Michael Ermitsch, Bassam Redwan, Olaf Schega, and Christian Biancosino

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The chapter was inadvertently published with an incorrect chapter title, which has now been corrected to read as "Outcome of Repeat Pulmonary Metastasectomy".

The updated version of this chapter can be found at
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