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ORIGINAL ARTICLE

Immunohistochemical Diversity of Endometrial Carcinoma and Their Implications in Prognosis: A Prospective Clinicopathological Study in a Tertiary Care Hospital of Eastern India.

Rajashree Pradhan[1] · Ranu Roy Biswas[2] · Sajeeb Mondal[3] · Upasana Mukherjee[4]

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Abstract

Background Endometrial cancer is the sixth most common cancer in women worldwide with gradually increasing incidence and mortality rate. The most recent classification of endometrial carcinoma (EC) with diagnostic flowchart includes both immunohistochemical and molecular markers for prognostic purpose and better management of endometrial cancer. In this study, we want to analyze various immunohistochemical (IHC) markers in EC and their prognostic significance.

Methods This was a prospective study conducted from August 2016 to February 2024. We studied 168 cases of EC for histopathological subtypes, grading and various IHC markers such as Estrogen Receptor (ER), Her 2 Neu, Cytokeratin 5/6, Epithelial Membrane Antigen (EMA) and p53.

Results In our study, most common histological subtype was endometrioid (132) followed by serous (17), mucinous (8), clear cell (7) and carcinosarcoma (4). ER expression was mostly seen in endometrioid type. Loss of ER expression and Her 2 expression along with p53 over expression was not only associated with high grade EC but also with advanced clinical stage and lymph node metastasis.

Conclusion Immunohistochemical markers play a definite role in risk stratification and specific individual oriented therapy in endometrial cancer patients.

Keywords Endometrial cancer · ER · Immunohistochemistry · p53

Introduction

According to the most recent GLOBOCAN 2020, Endometrial cancer (EC) is the sixth most common cancer in women worldwide with 417,000 new cases and 970,000 deaths in 2020 [1, 2]. Over recent years there is increase in both EC incidence and mortality rates in several Asian countries. In 2020, the number of new cases of EC was 16,413 in India which was much higher in comparison to other Asian countries such as 775 cases in Singapore, 1401 cases in Malayasia and 3425 cases in South Korea. [3, 4]. Areas of the World

Rajashree Pradhan, MD Pathology, Associate Professor; Ranu Roy Biswas, MD Pathology, Professor; Sajeeb Mondal, MD Pathology, Associate Professor; Upasana Mukherjee, MD Pathology, Senior Resident.

✉ Upasana Mukherjee
mupasana@rediffmail.com

Rajashree Pradhan
pradhanrajashree99@gmail.com

Ranu Roy Biswas
ranuroybiswas1968@gmail.com

Sajeeb Mondal
sjbmondal@gmail.com

[1] Department of Pathology, College of Medicine and Sagore Dutta Hospital, Kolkata, West Bengal, India

[2] Department of Pathology, Medical College Kolkata, Kolkata, West Bengal, India

[3] Department of Pathology, Rampurhat Government College and Hospital, NH14Rampurhat, Birbhum, West Bengal 731224, India

[4] Department of Pathology, College of Medicine and Sagore Dutta Hospital, Kolkata, West Bengal 700058, India

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with high Human Development Index (HDI) had three fold more incidence of EC in comparison to the areas with low or medium HDI [5].Several features of EC such as histological subtype, FIGO stage, histologic grade, presence of lympho vascular invasion (LVSI) and depth of myometrial invasion serve as prognostic markers of EC [5]. As per a research paper by Cancer Genome Atlas Research Network [6], there are four molecular categories of EC based on prognosis and response to post surgical adjuvant therapy [7]. In clinical practice, molecular classification is difficult to implement because of high costs and need of frozen tumor tissue for molecular studies. Due to this, diagnostic algorithms including both molecular tests and immunohistochemical markers where formalin-fixed, paraffin-embedded tumor tissue can be used, have been proposed and tested [8]. The most recent classification of endometrial cancer with diagnostic algorithms including both immunohistochemical markers and molecular markers was proposed by European Society of Gynecology Oncology/Radiotherapy/Pathology [9]. This guidelines has better explained the ECs for prognosis as well as for management of endometrial cancer.

In this study, we aimed to analyze different immunohistochemical markers in endometrial cancer and association of these markers with that of clinicopathological features including survival of endometrial cancer patients.

Materials and Methods

This was a prospective study conducted from August 2016 to February 2024 at a tertiary care hospital in West Bengal, India. A total of 168 cases of EC patients were evaluated immunohistochemically and studied after considering the inclusion and exclusion criteria. Patients with endometrial cancer who underwent surgery in our institution and had follow-up data available were included in this study. Endometrial cancer patients whose follow-up data were not available were excluded from the study. All the clinical data such as age, body weight (BMI), parity, preoperative imaging findings, and postoperative therapy (chemo & radio) details were obtained from the clinical records.

All the tumor specimens received in our department were subjected to primary fixation with formalin and then grossing was done according to CAP (College of American Pathology) protocol. The grossed tissue were subjected to routine tissue processing and Hematoxylin & Eosin stained slides were prepared. All the slides from endometrial cancer tissue were examined by two expert histopathologists for confirmation of the diagnosis, histologic subtyping, pathologic staging, tumor grading, myometrial invasion and presence of lymphovascular invasion. Tumor subtyping was done according to recent WHO classification of tumors of FGT (5th Edition).[10] Tumor staging was done according to 8th edition of AJCC. The IHC staining for ER, Her 2 Neu,CK(5/6), p53 were done according to standard protocol. 3–4 µm sections were cut from the selected blocks of each tumor specimen and placed in Poly-L-lysine coated slides. Then, after deparaffinization on hydrated with graded alcohol, antigen retrieval was done by heating. Finally, the microsections were incubated with primary antibody followed by secondary antibody with specified clone and dilution as detailed below. Then 3,3'Diaminobenzidine (DAB)or 3-Amino-9 Ethyl carbazole (AEC) was added as chromogen. Till thus every step was followed by rinsing with wash buffer. Finally, counterstained with hematoxylin followed by mounting. Primary antibody clone and dilution for ER, Her2 Neu,CK (5/6) & P53 was(EP1,1:50),(Pg,636,1:150)(A0485,1:800)(D5/16B4,1:200) (Do-7,1:200) respectively. Nuclear staining for ER, PR& p53, membrane staining for Her 2 Neu and cytoplasmic staining for CK were considered positive when extent of immunostaining was > 10% of tumor cells.

All the data were expressed in numbers and percentage using software IBM SPSS 20.0.*P* value < 0.05 was considered statistically significant.

Results

In our study, 102 cases (60.71%) were above 60 years. Predominant histologic subtype was endometrioid (132,78%)((Fig. 1a,b)(Fig. 2a) along with serous carcinoma(17%,10.11%)(Fig. 2b), non-intestinal mucinous (08,4.76%)(Fig. 1c), clear cell carcinoma (07,4.16%) (Fig. 2c) and carcinosarcoma (4,2.38%)(Fig. 2d) as other histologic subtype. Out of 168 cases low-grade and high-grade tumors were 105 (62.5%) and 63(37.5%) respectively with 99 cases (58.92%) had stage (I & II) and 69 cases had stage (III & IV) (Table 1). grade endometrioid Out of different IHC markers studies ER (Fig. 3a) & CK 5/6 (Fig. 3b) expression most commonly associated with the endometrioid subtypes and Her 2 (Fig. 3c) and p53 (Fig. 3d) expression was mostly seen in high grade endometrioid, serous, clear cell and carcinosarcomas(Table 2).

In our study, loss of ER expression and p53 expression was commonly associated with important prognostic markers such as lymph node metastasis and advanced clinical stage(Table 3).

Discussion

Adenocarcinoma of the endometrium also known as EC or carcinoma of the uterine corpus is the most common malignancy of the female genital tract [11]. With an estimated 65,950 new cases and 12,550 deaths result from this

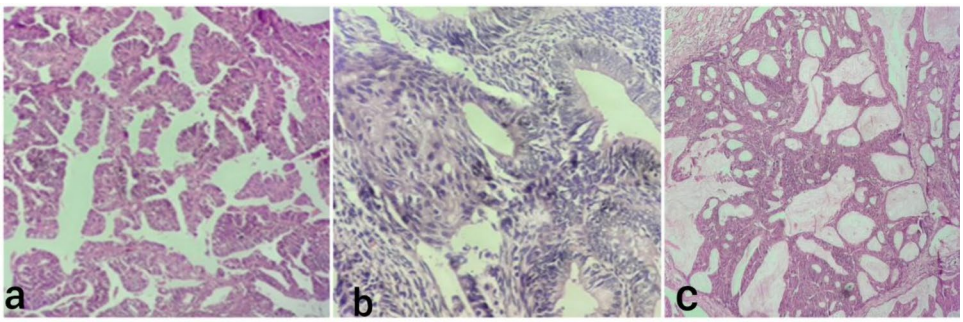

Fig. 1 Low grade endometrioid carcinoma [grade 1 and grade2 (**a** & **b**)] and low grade non intestinal mucinous carcinoma(**c**) (H&E,100X)

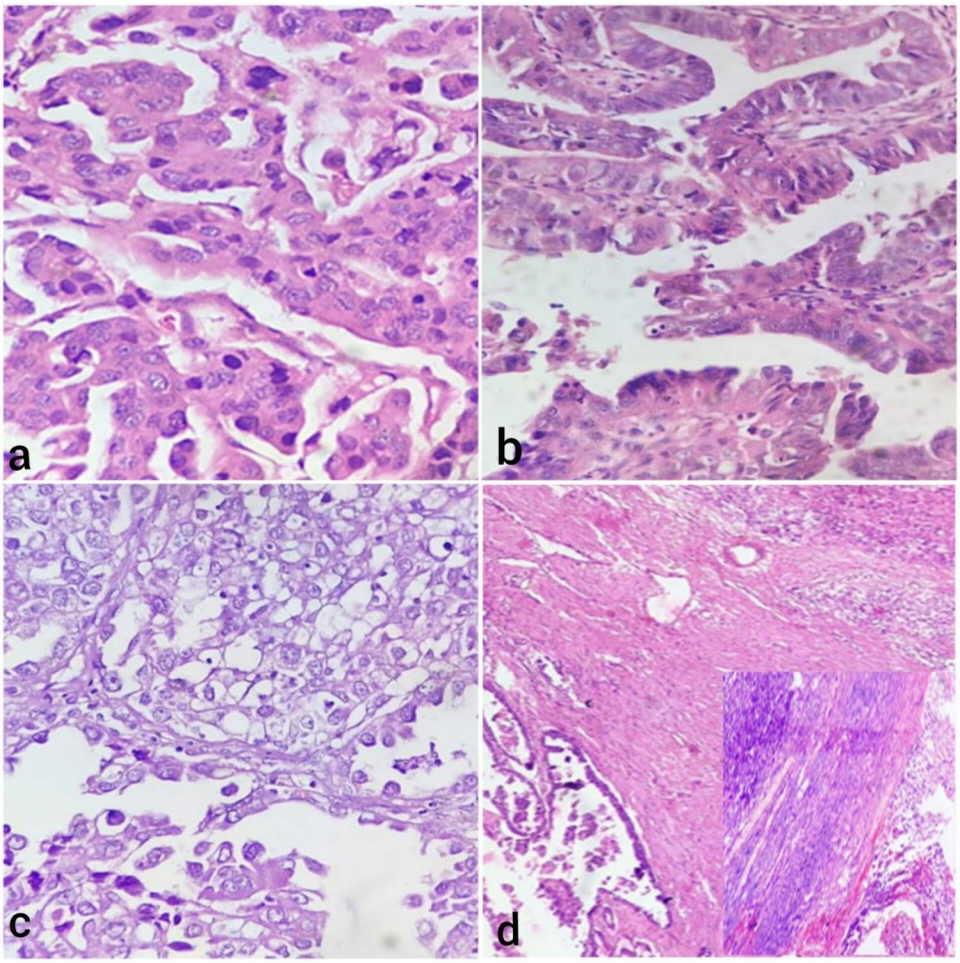

Fig. 2 High grade endometrial carcinoma [grade 3 endometrioid carcinoma(**a**),serous carcinoma(**b**),clear cell carcinoma(**c**),carcinosarcoma(**d**)] (H&E,100X)

cancer. According to the International Agency for research on cancer, the incidence rate of endometrial cancer is increasing rapidly and is going to increase by more than 50% worldwide by 2040 [12]. High income countries have a greater incidence of endometrial cancer (11.1/100000 females) [13] compared with low income countries (3.3 per /100,000 females) which might be due to high rates of obesity, physical inactivity and extended life expectancy. Elevated estrogen levels especially in postmenopausal obese women known to be the most significant cause of the increased risk of endometrial cancer. Estrogen response is a key oncogenic pathway in endometrial cancer development [14]. On contrary, mortality caused by endometrial cancer is highest among women of low socioeconomic

Table 1 Clinicopathological features of patients with endometrial carcinoma

	Parameter	Numbers	Percentage(%)
1	Age <60 Years >60 years	66 102	39.28 60.7
2	Histologic Type Endometrioid Serous Carcinoma Non intestinal mucinous Clear cell Carcinoma Carcinosarcoma	132 17 08 7 4	78 10.11 4.76 4.16 2.38
3	Histologic Grade Low grade High grade	105 63	62.5 37.5
4	LVSI Yes No	54 114	32.14 67.85
5	Myometrial Invasion Not present/<50% >50%	119 49	70.8 29.1
6	Cervical involvement Yes No	66 102	39.28 60.71
7	Regional lymph node Not submitted Submitted	98 70 46(negative) 24(positive for tumor deposit)	58.3 33.3 8.3
8	FIGO stage Stage (I &II) Stage (III & IV)	99 69	58.92 41.07

n = 168

status due to the inability of getting standardized treatment [15]

Endometrial cancer has many histological subtypes. In our study, all the endometrial cancers were categorized according to the latest WHO Classification of female genital tract. [10]. The major types of endometrial cancer according recent WHO classification includes the following subgroups: endometrioid, serous, clear cell, mixed cell adenocarcinoma and other relatively rare types including mucinous adenocarcinoma, neuroendocrine tumors, dedifferentiated carcinoma and undifferentiated carcinoma.

Histologic grading of EC is done using the 3 tier grading system by FIGO [16] only for endometroid and non-intestinal type mucinous carcinomas as follows.

Grade 1: 5% or less non squamous solid growth pattern.

Grade 2: 6% to 50% non squamous solid growth pattern.

Grade 3: > 50% non squamous solid growth pattern.

The latest FIGO 2023 revisions [17] adopted the WHO two- tier system (low vs high) for grading endometroid and non intestinal mucinous carcinomas.

Low grade: Grade1 & 2 of(endometroid and non intestinal mucinous carcinoma).

High grade: i. Grade 3 (FIGO) of endometrial & non intestinal mucinous carcinoma.

ii. Uterine Serous Carcinoma.

iii. Clear cell Carcinoma.

iv. Mixed adenocarcinoma.

v. Dedifferentiated and undifferentiated carcinoma.

In our study, histologic grading was done according to the recent binary system of WHO adapted by FIGO (Table 1) (Fig. 1,2).

pathological staging was done following (p TNM) AJCC (8th edition) classification and clinical staging by FIGO [17].

In 2020, the World Health Organization recommended the molecular classification of EC using surrogate markers and following a standardized diagnostic algorithm [18]. This approach categorizes EC into four molecular classes (1) POLE (polymerase epsilon) ultra mutated (2) Microsatellite instability hypermutated (3) Copy number low and (4) Copy number high [19].

Our study demonstrated that the immunophenotype of endometrial cancers are diverse, in distribution and also in intensity of expression with each markers.

ER:ER expression are known to be common in well differentiated (grade1 & 2) endometrioid carcinoma of endometrium. Several retrospective studies done earlier support that ER and PR are independent prognostic markers in primary tumors. In our study, ER expression was mostly seen in low grade endometrioid carcinoma (Fig. 3a) with few high grade tumor also showed ER positivity. The reduced expression of ER in patients of EC could suggest grade 3 (high grade) cancer as reported by Przewozny et al. [20]. Similarly in our study negative ER is associated with higher grade of EC (Table 3). In our study, negative ER was associated with higher clinical stage of EC similar to the study by Wang et al. [21] (Table 3).

Her 2:Her 2 overexpression has been established as an important biomarker with both therapeutic and prognostic implications in breast and gastric cancer. Her 2 protein overexpression and/or ERBB2 amplification have also been described in EC. Due to lack of an universal Her 2 Neu testing and scoring methods and difference in histological subtypes included in the previous studies the exact prognostic significance in EC was not established in the past. Nevertheless, there is established evidence that points towards correlation between Her 2 expression with serous EC [22]. Recently, a Phase II clinical trial showed increased disease Progression free survival in favor of combined chemotherapy and Trastuzumab therapy in Her 2 positive endometrial cancer [23]. Her 2 expression also analyzed with p53 overexpression on the basis of molecular classification of EC [24]. In our study, Her 2 overexpression mostly associated with

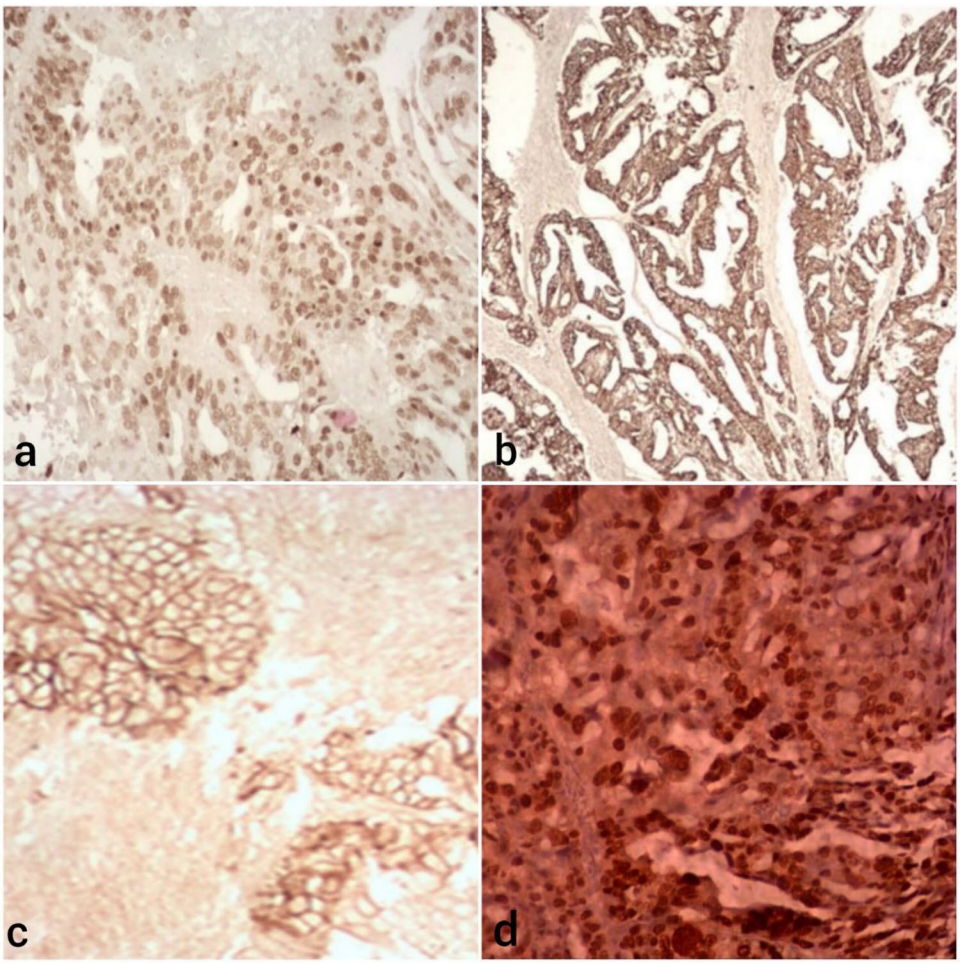

Fig. 3 Different IHC markers expression in low grade endometrial carcinoma: ER (**a**),CK (**b**) and high grade EC:Her 2 Neu (**c**), p53(**d**) (IHC,ER,CK5/6,Her 2 neu,P53 100x)

Table 2 ER, Her2 Neu, EMA, CK5/6, p53 expression in endometrial carcinoma

Lesion Type (Number)	ER Number (%)	Her 2 Neu Number (%)	EMA Number (%)	CK5/6 Number (%)	p53 Number(%)
Endometrioid carcinoma (132)	127 (96.2%)	18 (13.63%)	13 (9.84%)	97 (73.48%)	17 (12.87%)
Mucinous Carcinoma (Non intestinal) (08)	5 (62.5%)	3 (37.5%)	1 (12.5%)	5 (62.5%)	1 (12.5%)
Uterine Serous Carcinoma (17)	0	15 (88.23%)	11 (64.7%)	4 (23.5%)	17 (100%)
Clear cell Carcinoma (7)	0	6 (85.57%)	3 (42.8%)	3 (42.8%)	7 (100%)
Carcinosarcoma(4)	1 (25%)	3 (75%)	1 (25%)	1 (25%)	4 (100%)

n = 168

serous and clear cell histology along with some endometroid carcinoma also showed positivity(Table 2,Fig. 3c).

CK (5/6): Cytokeratin are the major subgroup of intermediate filaments encoded by Keratin genes. CKs have been developed as one of the prognostic indicators in various epithelial malignancies. Immunohistochemical detections of CKs have become an important tool in clinical tumor pathology [25].CK profiling is especially useful for poorly-differentiated carcinoma, widespread carcinoma involving many organs especially in carcinoma of unknown primary [26].In EC, CK is useful to identify small clusters of cancers cells in lymph node micro metastasis which is a ‘’gold’’ standard

Table 3 Comparison of loss of ER expression and p53 overexpression with prognostic parameters of endometrial carcinoma

Prognostic parameters	Loss of ER	p53 expression	p value
High grade EC(63)	57(90.4%)	63(100%)	< 0.001
Advanced stage(28)	28(100%)	28(100%)	< 0.001
Lymph node metastasis(24)	24(100%)	24(100%)	< 0.001

procedure [25, 27]. The presence of CK5/6 immunostaining was more frequently seen in endometrioid type of carcinoma and loss of CK5/6 expression in ECs was frequently associated with a higher FIGO stage [27]. In our study, CK5/6 immunostaining was mostly seen in endometrioid type of EC (Table 2, Fig. 3b).

EMA: EMA overexpression is a useful marker of endometrial malignancies. According to recent studies, high grade EC showed diffuse positivity for EMA [25]. In our study, most of the endometrioid carcinoma showed intense diffuse staining (Tables 2 and 3).

p53: As mentioned earlier p53 IHC is an important parameter in the diagnostic algorithm for molecular classification and prognostic evaluation of EC [19]. The correct interpretation of p53, IHC is crucial because it significantly affects a patient's individual risk assessment and subsequent management [9]. p53 overexpression is consistently being linked with higher grade EC with serous and clear cells histology. In our study, p53 overexpression is mostly seen in high grade and higher clinical stage of EC (Table 3, Fig. 3d). Recent literature suggests that patients with p53 overexpression (high risk) benefit from the addition of chemotherapy to adjuvant treatment regimen [19]. It is also recommended to use combined adjuvant radiotherapy with chemotherapy even for patients with stage I-IV with p53 overexpression according to ESGO-ESTRO-ESP EC guidelines [9].

Conclusion

To conclude, in our study, we demonstrated loss of ER expression and p53 expression is not only associated with high grade EC but also with poor prognostic markers such as lymph node metastasis and advanced clinical stage. The different immunohistochemical profiles of endometrioid and serous carcinomas confirm different molecular pathways in their development. Immunohistochemical markers play a vital role in differentiating women with EC into homogeneous prognostic group leading to specific individualized treatments. Routine use of biomarkers testing in EC may be recommended to tailored endometrial cancer treatment.

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Author contribution Dr.Rajashree Pradhan helped in concept, design, methodology and original draft. Dr Ranu Roy Biswas done data collection and supervision. Dr Sajeeb Mondal contributed to supervisions, formal analysis and investigation. Dr Upasana Mukherjee helped in review, editing and final draft preparation.

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Declarations

Conflict of interest There are no potential conflicts of interest to declare.

Ethical Approval This study was approved by the Institutional Ethics committee before initiation of the study (scanned copy attached) along with the Certificate of completion given. (**ID: ECR/1210/Inst/WB/2019/RR-22)** (scanned copy attached).

Human and Animal Rights Research involved human participants for immunohistochemical analysis of endometrial cancer patients and no animal trials were involved.

Informed consent Written informed consent was obtained from all the patients.

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