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ORIGINAL ARTICLE

The Role of Serum Galactomannan Assay as a Potential Surrogate Biomarker for Fungal Microinvasion in Allergic Fungal Rhinosinusitis

Anupam Kanodia[1] · Ashu Seith Bhalla[2] · Gagandeep Singh[3] · Immaculata Xess[3] · Bashid Valia Valappil[1] · Aanchal Kakkar[4] · Shilpi Budhiraja[1] · Kapil Sikka[1] · David Victor Kumar Irugu[1] · Alok Thakar[1] · Hitesh Verma[1]

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Abstract We conducted this study to determine if serum galactomannan (GM) can be used as a marker to implicate the invasiveness of allergic fungal rhinosinusitis (AFRS), and correlate this value with the aggressiveness of disease documented via computed tomography (CT). All paranasal CT scans done for AFRS patients prospectively over a five-year period (2015–2019) were included. An indigenous 20-point score was used to document the extent of bone erosion seen on CT, wherein a higher score meant a greater extent of bone erosion. It was then correlated with serum GM scores. The median CT scores of galactomannan-positive (GM+) patients were compared with the median CT scores of galactomannan-negative (GM−) patients 3 using Mann–Whitney U test. The patients were divided into five groups based on the extent of disease-No bone erosion, erosion of only sinus wall/orbit, 3 erosion of orbit and skull base, erosion of only skull base and lateral extension of disease into infratemporal fossa (ITF). Subgroup analysis was conducted over mean GM values in these groups using ANOVA test. p-value < 0.05 was considered significant. Statistical analysis was performed using SPSS version 25.0. A total of 92 patients were included (56 males, 36 females). No statistically significant difference was found (p-value $= 0.42$) between the CT scores of galactomannan-positive (GM+) group and galactomannan-negative (GM−) group. The mean GM scores amongst the five sub-groups did not show a statistically significant difference. Serum galactomannan values correlate poorly with aggressiveness of disease quantified on non-contrast CT of paranasal sinuses.

Keywords Fungal sinusitis · Rhinosinusitis · AFRS

Introduction

Allergic fungal rhinosinusitis (AFRS) is a variant of chronic rhinosinusitis with nasal polyps (CRSwNP) with type I and III hypersensitivity reactions to fungal elements, often with superadded bacterial infections and increased vascularity. Though gradually progressive, the pressure symptoms of the disease may cause complications like vision impairment and skull base erosion [1]. AFRS is managed with surgical clearance of disease & steroid therapy, both systemic and topical. Though considered non-invasive, Thakar et al. [2] have shown mucosal invasion by fungal hyphae in AFRS, predominantly in patients with intracranial or intra-orbital extension of disease. This tendency to invade tissues might be a cause of surgical failure and recurrence of disease. Hence, it is imperative to document this invasion and start an adjuvant treatment to prevent multiple surgeries.

Aspergillus is the most common species causing AFRS especially in South-East Asia, and serum galactomannan (GM) serves as a surrogate marker to assess tissue invasion. Galactomannan is a one of the constituents of fungal cell wall of Aspergillus [3]. The serum assay utilizes ELISA test to detect circulating GM. A biopsy from the representative site is the ideal method to document tissue invasion, however it is not always feasible to pinpoint the representative area

✉ Hitesh Verma
drhitesh10@gmail.com

1 Department of Otorhinolaryngology and Head-Neck Surgery, Room no 4057, All India Institute of Medical Sciences, Ansari Nagar, New Delhi, Delhi 110029, India

2 Department of Radiodiagnosis, All India Institute of Medical Sciences, New Delhi, India

3 Department of Microbiology, All India Institute of Medical Sciences, New Delhi, India

4 Department of Pathology, All India Institute of Medical Sciences, New Delhi, India

based on radiology owing to the tendency of this disease to cause thinning of bone. Furthermore, subjective variation in radiological interpretation and lack of representative biopsy specimen can lead to inaccurate assessment of mucosal invasion of the disease. Thus, there is a possible role of serum GM assay to quantify the mucosal invasion, and hence, theoretically, serum GM values should serve as a surrogate marker for tissue invasion.

In other forms of invasive rhinosinusitis, like acute invasive fungal rhinosinusitis (AIFR), serum GM has been used as a surrogate marker. It is found to be elevated in AIFR and has been used to indicate the fungal species and to predict clinical outcomes [4, 5]. Serum GM is also used in cases of pulmonary aspergillosis to titrate the treatment. We conducted this study on the premise that AFRS shows patchy mucosal invasion at places and thus, serum GM can be used as a marker to implicate invasiveness of AFRS and correlate this value with the aggressiveness of disease documented via computed tomography (CT).

Methods

This was a prospective cohort study conducted at our tertiary care centre over a period of five years (2015–2019) which included all patients of AFRS who presented to us. A written informed consent was obtained from all the participating patients. Institutional ethical clearance was obtained. The diagnosis of AFRS was established by correcting the clinical, radiological, microbiological & histopathological findings. We evaluated the extent of the disease using their pre-operative non-contrast CT (NCCT) scans of paranasal sinus (PNS) and orbit. Each CT was given a score based on the 20-point CT scoring system (Table 1), to objectively assess the aggression of disease. This system scores any evidence of bony erosion, which may be a pointer for mucosal invasion. This scoring system was adopted as Lund-Mckay score does not include peri-sinus wall erosion in its scoring system [6]; and AFRS frequently presents with erosion of peri-sinus walls which insinuates tissue invasion. We evaluated if these scores would correlate with serum GM level.

This CT score was then correlated with the pre-operative serum GM levels. Patients' whose data lacked either the CT findings or the serum GM levels were excluded from the study. Serum GM level of >0.5 was treated as positive. Spearman's rank correlation coefficient (ρ) was then used to quantify the correlation between both the variables. Patients who had taken penicillin treatment for any indication in preceding two weeks of enrolment in the study were excluded as it has been known to affect serum galactomannan values. The surgically unfit patients were also excluded from the study. The median CT scores of galactomannan-positive (GM+) patients were compared with the median CT scores of galactomannan-negative (GM−) patients using Mann–Whitney U test.

Table 1 The proposed scoring system to quantify fungal invasion on computed tomography (CT)

S. No.	Anatomical location	Scoring
1	Nasal cavity	0-absent, 1-present
2	Osteomeatal complex	0-absent, 1-present
3	Ethmoid sinus	0-absent, 1-present
4	Septum erosion	0-absent, 1-present
5	Hard palate erosion	0-absent, 1-present
6	Frontal sinus	0-absent, 1-present
7	Anterior wall of frontal sinus erosion	0-absent, 1-present
8	Posterior wall of frontal sinus erosion	0-absent, 1-present
9	Sphenoid sinus	0-absent, 1-present
10	Sphenoid sinus lateral wall erosion	0-absent, 1-present
11	Sphenoid sinus roof erosion	0-absent, 1-present
12	Sphenoid sinus posterior wall erosion	0-absent, 1-present
13	Lamina erosion	0-absent, 1-present
14	Cribriform plate	0-absent, 1-present
15	Fovea ethmoidalis erosion	0-absent, 1-present
16	Planum sphenoidale	0-absent, 1-present
17	Maxillary sinus	0-absent, 1-present
18	Maxillary sinus anterior wall erosion	0-absent, 1-present
19	Maxillary sinus posterior wall erosion	0-absent, 1-present
20	Pterygoid plates	0-absent, 1-present

Further, the patients were divided into five groups based on the extent of disease- No bone erosion, erosion of only sinus wall/orbit, erosion of orbit and skull base, erosion of only skull base and lateral extension of disease into infratemporal fossa (ITF). The mean serum galactomannan scores of these groups were compared with each other and checked for any significance using ANOVA test. p-value <0.05 was considered significant. SPSS version 25.0 was used to perform statistical analysis.

All enrolled patients underwent endoscopic clearance of disease. The operating surgeons were instructed to document and biopsy the suspected area of the tissue invasion for histopathological documentation. The patients were kept under regular follow-up.

Results

A total of ninety-two patients with AFRS could be enrolled. The clinical, demographic, radiological and biochemical details of the patients have been tabulated in Table 2. All these patients underwent endoscopic clearance.

The Spearman's rank correlation coefficient was calculated to be $r_s = -0.17037$ which meant that there was no

Table 2 Demographic and clinical characteristics of the patient population

Characteristics	Number of patients, n (%)
Sex	
Male	56
Female	36
Age (in years)	
Median ± SD	30 ± 14.48
Ophthalmological symptoms due to disease	
Proptosis	2
Vision impairment	2
CT scores	
Mean ± SD	10 ± 5
Median (IQR)	11 (6)
GM scores	
Score > 0.5	26
Median (IQR)	0.39 (0.29)
Mean ± SD	0.45 ± 0.24

significant correlation between the CT scores and serum GM values.

No statistically significant difference was found (p-value = 0.42) between the CT scores of galactomannan-positive (GM+) group and galactomannan-negative (GM−) group (Table 3).

Subgroup analysis was performed with the five groups as shown in Table 4.

The fifth group was excluded from the statistical analysis due to a small dataset. The other four groups were analysed. The f-ratio value on ANOVA test was 1.23146 and calculated p-value was 0.303. Hence the result was not found to be statistically significant. Tissue invasion was not noticed intraoperatively & in histopathology specimen in any patient. The follow-up period ranged from 6 to 39 months. No patient underwent revision surgery or developed any complication due to the disease.

Table 3 Comparison of the median CT scores between the GM+ and GM− group

Group	Median (IQR) CT score
GM+ group (26 patients)	9 (7.5)
GM− group (66 patients)	12 (6)

Table 4 Comparison of CT scores and serum galactomannan values amongst different groups formed based on disease extent

Characteristics	Number of patients, *n*	Mean serum galactomannan value (± SD)
No bone erosion	52	0.44 ± 0.20
Only sinus wall/orbit erosion	12	0.5 ± 0.34
Orbit + skull base erosion	12	0.48 ± 0.25
Skull base erosion	14	0.34 ± 0.17
Lateral extension into infratemporal fossa	2	0.98

Discussion

As per our knowledge, this is the first study which has looked at serum galactomannan as a potential tool to document micro-invasion in AFRS. We found no correlation between bone erosion documented on CT scores and the serum GM values. Out of 92 subjects, 26 had high GM values (0.5); however, there was no proportionate increase in CT scores that was documented. Most of the published literature from the Indian subcontinent implicates Aspergillus species as the most common causative organisms for AFRS [7, 8]. Hence, an elevated value of serum galactomannan is expected in the presence of tissue invasion. However, despite CT being suggestive of tissue invasion, we could not find any tissue invasion histopathologically which explains discordant serum GM values. Hence, CT scores also did not correlate with serum values as these scores overestimated the extent of bone erosion.

On performing sub-group analysis, we could not find any significant difference in the serum GM values between various groups divided based on the site of bone erosion. Neither the groups with orbital bone erosion (lamina papyracea or orbital floor) nor the groups with skull base erosion had a significantly elevated serum GM values. Again, this can be attributed to over-estimation of bone erosion by CT. MRI is also a poor modality to evaluate bone erosion; it, however, is specific in detecting tissue invasion. Two patients were detected to have erosion of posterior maxillary sinus wall with extension of disease in the infratemporal fossa. They had serum GM scores of 0.54 and 1.42 respectively. The disease was removed completely, and histopathological examination did not show tissue invasion. We cannot comment on the significance of serum GM values in these two patients.

Some authors have explored serum GM assay's utility in AIFR. Tong et al. [9] commented that serum GM assay should only assist the diagnosis of invasive aspergillosis as the test results were not sensitive or specific enough. While working on AIFR patients, Cho et al. [4] detected significantly elevated GM levels in invasive aspergillosis patients when compared to invasive mucormycosis patients and

patients with fungal ball. Melancon et al. [10] have shown the role of serum GM assay in AIFR; however, there is limited literature on the utility of this investigation in AFRS. Kostamo et al. [11] in their study on 25 subjects with CRSwNP, also did not report any utility for serum GM assay in AFRS. Our study supports the existing literature that there is limited role of this assay in AFRS, possibly as the extent of invasion is much less as compared to invasive pulmonary aspergillosis or acute invasive fungal sinusitis. Also, the invasion is over-estimated by CT and hence one should not label the disease as invasive until proven histopathologically.

The study had some limitations. As AFRS is a diffuse disease with multiple areas of focal microinvasion, it is often a futile exercise to document histopathological mucosal invasion. It can be seen in Fig. 1 (coronal sections) and Fig. 2 (sagittal sections) how multiple points of erosion can be seen in a single patient. We evaluated the non-contrast CT (NCCT) in detail using our scoring system to check for areas of erosion. Another limitation of the study is that we have taken erosion as a marker of mucosal/soft tissue invasion on HRCT. Extra-sinus extension, which is a better marker for tissue invasion would require a contrast-enhanced magnetic resonance imaging (CEMRI) or at least a contrast-enhanced computed tomography (CECT). Also, due to thinning of bones caused by the expansive disease, CT can often be over-sensitive for bone erosion, and hence has poor specificity. Lastly, we did not check for GM levels in the mucin or nasal wash samples obtained during surgery, which could have pointed towards a diagnosis of invasive sino-nasal aspergillosis.

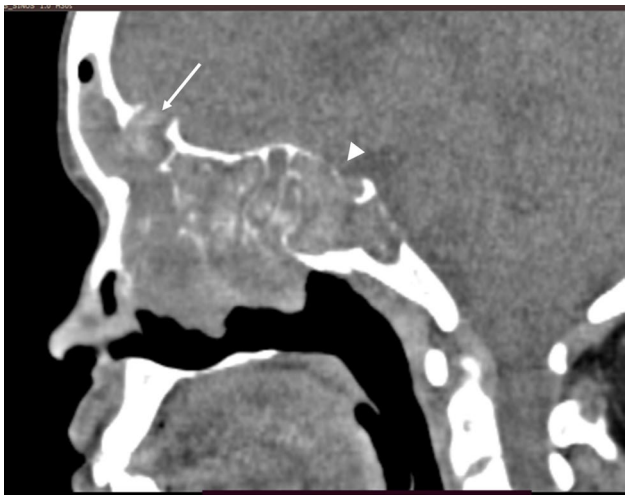

Fig. 2 Non-contrast computed tomography (CT) sagittal section, showing posterior wall of frontal sinus erosion (white arrow) and sphenoidal roof erosion (arrowhead)

Conclusion

Serum galactomannan values correlate poorly with aggressiveness of disease quantified on CT of PNS. We could not find a statistically significant correlation between the two despite Aspergillus being the most common cause of AFRS. Further studies incorporating CEMR or CECT in their protocols are needed to document tissue invasion with AFRS and its statistical correlation with serum galactomannan values.

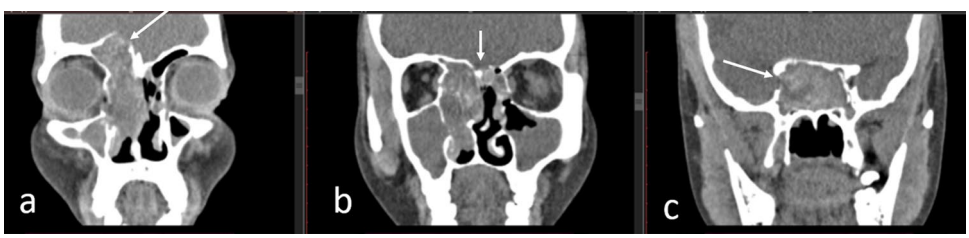

Fig. 1 Non-contrast Computed tomography (CT) coronal sections of paranasal sinuses for allergic fungal rhinosinusitis. This CT shows right fovea ethmoidalis erosion (**a**), left cribriform plate erosion (**b**) and right lateral sphenoidal wall erosion (**c**) (All findings depicted by arrows)

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Declarations

Conflict of interest The authors declare that they have no conflict of interest to disclose.

Ethical approval An informed written consent was obtained from all participating patients included in the study. The study was 'approved from ethical angle prospectively w.e.f. 18 March 2017' by the institutional ethics committee. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institution and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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