Received Date : 29-Jan-2015

Revised Date : 03-Jun-2015

Accepted Date : 08-Jun-2015

Article type : Manuscripts

Chronic Mastitis in Egypt and Morocco: differentiating between idiopathic granulomatous mastitis and IgG4-related disease

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Short Title: Chronic Mastitis in Egypt and Morocco

Key Words: Mastitis, IgG4-related disease, Idiopathic granulomatous mastitis

Abstract

Idiopathic granulomatous mastitis (IGM) is a benign, frequently severe chronic inflammatory lesion of the breast. Its etiology remains unknown and reported cases vary in their presentation and histologic findings with an optimal treatment algorithm yet to be described owing mainly to the disease's heterogeneity. IgG4-related disease (IgG4-RD) is a newly recognized systemic fibroinflammatory condition characterized by a dense lymphoplasmacytic infiltrate with many IgG4-positive plasma cells, storiform fibrosis, and obliterative phlebitis. Immunosuppressive therapy is considered to be an effective first-line therapy for IgG4-RD. We sought to clarify and classify chronic mastitis according to the histologic findings of IgG4-RD mastitis with respect to IGM and to develop a robust diagnostic framework to help select patients for optimal treatment strategies. Using the largest collection to date (43 cases from Egypt and Morocco), we show that despite sharing many features, IGM and IgG4-RD mastitis are separate diseases. To diagnostically separate the diseases, we created a classification schema – termed the Michigan Classification – based upon our large series of cases, the consensus statement on IgG4-RD, and the histologic description of IGM in the literature. Using our classification, we discerned 17 cases of IgG4-RD and 8 cases of IGM among the 43 chronic mastitis cases, with 18 indeterminate cases. Thus our Michigan Classification can form the basis of rational stratification of chronic mastitis patients between these two clinically and histopathologically heterogeneous diseases.

Introduction

Idiopathic granulomatous mastitis (IGM) is a non-neoplastic, chronic inflammatory lesion of the breast that mimics carcinoma both clinically and radiologically (1-5). IGM affects mostly parous women of child-bearing age, but has been reported in the age range of 11-80 years (2, 6). Patients most commonly present with an enlarging, firm, and tender breast lump with erythema and occasionally nipple retraction and/or axillary lymphadenopathy (3, 4, 7, 8). With these concerning clinical characteristics and non-specific imaging findings frequently resembling inflammatory breast cancer, the diagnosis is often made by core needle biopsy (1-4, 6, 7, 9). Histologically, IGM appears as non-caseating granulomas, frequently centered on the breast lobules, with epithelioid histiocytes and multinucleated giant cells and varying numbers of plasma cells, lymphocytes, neutrophils, and eosinophils (8, 10). IGM remains a

diagnosis of exclusion, however, as there are multiple processes that may cause granulomatous inflammation of the breast (8). An optimal treatment algorithm remains elusive owing mainly to the lack of a complete etiological classification. Therapeutic choices comprise observation, immunosuppressive therapy, wide local excision, mastectomy, or combined therapies; however, these strategies have varied success and recurrences are common (2, 6, 7, 9, 11).

IgG4-related disease (IgG4-RD) is a newly recognized fibroinflammatory condition affecting various organs (12-14). It is characterized by a dense lymphoplasmacytic infiltrate with many IgG4-positive plasma cells, storiform fibrosis, obliterative phlebitis, and often elevated serum IgG4 concentration (12-17). Reportedly uncommon are the presence of well-formed granulomas and a marked neutrophilic infiltration (12, 13, 18). IgG4-RD is now known to at least partly explain a broad range of medical conditions previously believed to be unique diseases (12, 15, 17). While no randomized clinical trials have been conducted, glucocorticoid treatment is the standard first-line therapy (12, 15).

In contrast to the abundance of cases in other organs, there have been up to now only 9 reported cases of IgG4-RD occurring in the breast (19-23). Among these cases of IgG4-RD mastitis, there is a great heterogeneity in the clinical and histopathological findings and apparent overlap with IGM characteristics. Consistent with this observation, IgG4-RD is known to have variations in histologic appearance depending on the specific organ involved (12-14, 17, 18). Furthermore, IGM itself is known to have a heterogeneous appearance and lacks a consensus on an optimal treatment protocol (2, 3, 6, 9, 11). Thus, we sought to clarify and classify chronic mastitis according to the histologic findings of IgG4-RD mastitis with respect to IGM from a large retrospective collection of cases from Egypt and Morocco, in order to better characterize and distinguish the subset of chronic mastitis patients with IgG4-RD who would benefit from immunosuppressive therapy.

Materials and Methods

Study Population

We performed a retrospective hospital-based study and identified 43 cases of chronic mastitis of unknown etiology from 5 hospitals in Morocco (L'hopital Ibn Rochd, Hassan II University and L'Hopital Ibn Toufail) and Egypt (Cairo University Medical School, Mansoura University Oncology Center, and the Tanta Cancer Center) (5). The average age of the patients was 36.9 years (SD 9.1, range 17-60). Cases were defined as any female patient with histopathological diagnosis of chronic mastitis of unknown etiology based upon an excisional biopsy and seen at the study hospitals from 2008-2011. All patients initially presented with a breast mass and routine clinical workup (e.g. acid-fast staining and appropriate cultures) was performed to rule out other common mass-forming and granulomatous-forming lesions of infectious and other etiologies. The only exclusion criterion was previous diagnosis of malignancy (5).

Further epidemiological and clinical characteristics (including lactation history and parity) of the study population are reported in detail in Oltean *et al* (5). The study underwent ethics board review and approval at all the institutions named above and at the University of Michigan.

Pathology Review and Dual Staining Protocol

Blinded to the IgG4 quantification, two authors (C.K. and S.G.A.) analyzed one haematoxylin and eosin (H&E) slide from each case sectioned from the same paraffin-embedded tissue cassette as were the immunostained slides. The parameters assessed were: a dense lymphoplasmacytic infiltrate, storiform fibrosis, obliterative phlebitis, epithelioid histiocytes, granulomas, giant cells, and neutrophils. Immunohistochemistry staining was performed as described previously (24). Specific to this study, slides were incubated for 1.5 hours at room temperature with an antibody cocktail containing rabbit anti-IgG (Cell Marque, Cat# 269A-15) diluted 1:5400 and mouse anti-IgG4 (Cell Marque, Cat# 367M-15) diluted 1:900 and detected with Mach 2 Double Stain 2, (Biocare Medical) for 10 minutes at room temperature.

Imaging and counting protocol

H&E images were captured using an Olympus BX41TF microscope with an Olympus UD03 CCD at 100x and 400x magnification. After immunostaining, digital images were captured by an Olympus DP26 CCD using an Olympus IX-51 microscope. The 200x immunostain digital images' field of view was 0.139 mm^2 in area. This was 20% smaller in area than the hpf reported by Cheuk *et al* (19). The three areas of the most intense inflammation were imaged and then IgG4 and IgG positive cells were quantified in each of the three images using ImageJ and averaged. The IgG4:IgG ratio was calculated as: IgG4/(IgG4+IgG).

Statistics

OriginPro 9.1 was used to calculate statistical differences using either Fisher's exact test or a t-test of unequal variances.

Results

Diagnosis of IgG4-Related Disease and Idiopathic Granulomatous Mastitis

Table 1 summarizes the histopathologic findings and IgG4 and IgG quantification of the cases. In total, 17 cases of IgG4-RD mastitis were identified along with 8 cases of IGM and 18 indeterminate chronic mastitis cases. In our schema, which we term the Michigan Classification, to be classified as most likely IgG4-RD mastitis, a sample must have met at least 4 of 5 positive criteria and 2 of 3 negative criteria. The positive criteria were adapted from and are consistent with the IgG4-RD consensus statement and the negative criteria were newly developed by our group to propose a classification schema that would encompass the heterogeneity of the findings in our large sample while in agreement with the consensus

statement and the general histologic characteristics of IGM described previously (10, 13). The positive criteria were: dense lymphoplasmacytic infiltrate, storiform fibrosis, obliterative phlebitis, >10 IgG4+ cells/hpf, and >40% IgG4:IgG ratio. The negative criteria were: epithelioid histiocytes, well-formed granulomas, and giant cells. Consistent with the histological description of IGM (10), cases were classified as IGM if they demonstrated: epithelioid histiocytes, vague or well-formed granulomas, and giant cells. The diagnostic criteria for IGM and our newly developed criteria for IgG4-RD mastitis are outlined in Table 2. The IGM category was assigned irrespective of the number of IgG4-positive plasma cells or IgG4:IgG ratio or the presence of other histologic characteristics of IgG4-RD. Although a prominent neutrophilic infiltration has generally been reported as relatively rare with IgG4-RD, this is known to vary by organ (12, 13). Neutrophils were not therefore considered in our diagnostic criteria for breast IgG4-RD because they did not appear to be a distinguishing characteristic between IgG4-RD mastitis and IGM in our large sample.

Pathologic Findings

Both IgG4-RD mastitis (Figure 1A) and IGM (Figure 1B) cases overwhelmingly had a dense lymphoplasmacytic infiltrate and only the indeterminate cases tended to have a mixed infiltrate. The presence of storiform fibrosis, however, was a more specific differentiator between the two diseases as it was present in 17 of 17 IgG4-RD mastitis cases (Figure 1C) and absent in the 5 of 8 IGM cases (Figure 1D) ($P<0.001$). Obliterative phlebitis was not a differentiating criterion and was seen in 11 of 17 IgG4-RD cases (Figure 1E) and present in 3 of 8 IGM cases ($P=0.39$). The presence of epithelioid histiocytes was the most common IGM feature overlapping with the IgG4-RD cases, being present in 5 of the 17 IgG4-RD cases. There were no well-formed granulomas in the IgG4-RD mastitis cases and giant cells were present in 3 cases. Representative images of IGM epithelioid histiocytes, granulomas, and giant cells can be seen in Figures 2A-D.

IgG4 Quantification

The average number of IgG4 cells per high power field and the IgG4:IgG ratio for each of the IgG4-RD mastitis, IGM, and indeterminate cases are plotted in Figures 3A-B. There were statistically significantly more IgG4+/hpf in the IgG4-RD cases than in the indeterminate cases ($P<0.01$), but not compared to the IGM cases ($P=0.061$). However, the average ratio of the IgG4-RD mastitis cases (49%) was significantly higher than the average ratio for both the IGM cases (26%, $P<0.001$) and indeterminate cases (20%, $P<0.001$). As has been reported previously (13, 14, 16, 17), an IgG4:IgG ratio of >40% proved quite specific as a diagnostic criterion with 14 of 17 IgG4-RD cases meeting the threshold, in contrast to only 2 of 8 IGM cases ($P<0.01$). Representative images of IgG4 and IgG dual-staining of IgG4-RD mastitis and IGM cases are shown in Figures 4 A-B and C-D, respectively.

Discussion

Our report describes the largest chronic mastitis series to date comprising of 43 cases from Egypt and Morocco and seeks to develop a robust framework to begin to differentiate and to understand the possible pathogenetic basis of this complex set of diseases. In light of previous work in this field, we first sought to understand IGM in the context of the more recently recognized IgG4-RD. Of these 43 chronic mastitis cases, 17 (40%) were determined to be IgG4-RD mastitis, 8 were IGM, and 18 were indeterminate cases of mastitis. Ogura *et al* (20) first posited that IGM could be separated into IgG4-related and non-IgG4-related cases, but our analysis supports the conclusion that the two appear more likely to be distinct disease entities. While the two cases of IGM Ogura *et al* (20) reported on did have significant numbers of IgG4 positive cells, an IgG4:IgG ratio was not calculated, which has proven to be the most specific indicator for IgG4-RD in our study and others (13). In our present study, when classified solely based upon the presence of epithelioid histiocytes, giant cells, and vague or well-formed granulomas, it is apparent that some of the IGM cases (see cases 18 and 22 Table 1) do share many features typical for IgG4-RD mastitis. Therefore, the distinction between the two diseases likely only became apparent with our larger sample size.

Furthermore, we were able to ascertain which histologic features are the most specific for differentiating between IgG4-RD mastitis and IGM by classifying IGM cases without regard to their IgG4-RD features. Therefore, by our schema, it was determined the most common and least specific IgG4-RD features seen in IGM were a dense lymphoplasmacytic infiltrate (7/8 cases) and large numbers of IgG4-positive plasma cells (5/8 cases). In contrast, the dual criteria of storiform fibrosis coupled with an IgG4:IgG ratio >40% is reasonably specific for IgG4-RD mastitis with 14/17 cases meeting both criteria. Additionally, since we did not specify which of the positive or negative criteria must be met to diagnose IgG4-RD mastitis, we can determine the most frequently absent IgG4-RD feature and most frequently present IGM feature. This is helpful in further clarifying the distinction between borderline cases. Obliterative phlebitis was the feature most often absent from IgG4-RD mastitis cases and epithelioid histiocytes the most common IGM characteristic seen in IgG4-RD mastitis. Therefore, the presence of giant cells or well-formed granulomas is quite specific within the breast for IGM when differentiating between it and IgG4-RD mastitis. Conversely, the absence of obliterative phlebitis should not preclude a diagnosis of IgG4-RD mastitis.

The average number of IgG4-positive plasma cells per high power field is lower for some of our reported IgG4-RD mastitis cases (12-29 cells/hpf for 7 of the 17 cases) than for cases reported in the literature (18, 19). We point to two likely explanations for this apparent deviation. First, our samples were biopsy samples known to have fewer IgG4+/hpf than surgical specimens (18) and, in fact, the consensus criteria lowers the requirement from 30 to 10 IgG4+/hpf for biopsy samples (13). All of our IgG4-RD mastitis

cases met this lower threshold. Second, we had an observable area of 0.139 mm^2 compared to 0.196 mm^2 reported by Cheuk *et al* (19) and mentioned by the consensus statement (13). While this area difference would not affect the IgG4:IgG ratio, it would be expected to affect the absolute number of positive cells. If the absolute counts for our IgG4-RD cases were multiplied by a 1.2x area scaling factor, then 13 of the 17 cases would have greater than 30 IgG4+ cells/hpf – even as biopsy and not surgical specimens. Ultimately though, it is the IgG4:IgG ratio that is a better and more specific diagnostic criterion for IgG4-RD.

In summary, we report on the largest chronic mastitis series to date comprising of 43 cases from Egypt and Morcco, of which 17 (40%) were IgG4-RD of the breast, 8 were IGM, and 18 indeterminate cases of mastitis. This also represents the largest collection of IgG4-RD of the breast – nearly doubling the number of previously reported cases (19-23). Among our samples, storiform fibrosis, an IgG4:IgG ratio >40%, and the absence of well-formed granulomas and giant cells is a highly specific 4-variable panel for IgG4-RD mastitis. IGM on the other hand is characterized by many giant cells and epithelioid histiocytes and one should not be misled by the occasional high number of IgG4-positive plasma cells or increased IgG4:IgG ratio. Thus our Michigan Classification requiring the presence of 4 of 5 positive criteria and absence of 2 of 3 negative criteria suggests a rational stratification basis between these two clinically and histopathologically heterogeneous diseases.

Acknowledgments

We would like to acknowledge financial support from the National Cancer Institute F30 fellowship program (NIH/NCI 1F30CA173910-01A1, SGA), the University of Michigan Rackham Predoctoral Fellowship (SGA), the Breast Cancer Research Foundation (SDM), the Avon Foundation (AS, SDM), and the Cancer Epidemiology Education in Special Populations Program (AS) (NIA R25 CA112383).

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Table 1

Table 2

IgG4-Related Disease Mastitis

Positive Criteria (4 of 5 present):	Negative Criteria (2 of 4 absent):
1. Dense lymphoplasmacytic infiltrate	1. Epithelioid histiocytes
2. Storiform fibrosis	2. Well-formed granulomas
3. Obliterative phlebitis	3. Giant cells
4. >10 IgG4 cells/hpf	
5. >40% IgG4:IgG ratio	

Idiopathic Granulomatous Mastitis

Positive Criteria (3 of 3 present):	Negative Criteria:
1. Epithelioid histiocytes	none
2. Vague or well-formed granulomas	
3. Giant cells	

Table/Figure Legends

Table 1 – Histopathology of Chronic Mastitis

The histopathologic characteristics and IgG4 and IgG immunostaining quantification of the IgG4-RD, IGM, and indeterminate cases.

Table 2 – Diagnostic criteria for IgG4-RD mastitis and IGM

The positive and negative criteria we developed to distinguish IgG4-RD mastitis from IGM.

Figure 1 – IgG4-RD and IGM comparison

Representative H&E images of IgG4-RD (**a**, **c**, and **e**) and IGM (**b** and **d**). A dense lymphoplasmacytic infiltrate is seen in IgG4-RD mastitis (**a**) and IGM (**b**), x400. (**c**) Storiform fibrosis in IgG4-RD mastitis, x100, x400. (**d**) Non-storiform fibrosis in IGM, x100, x400. (**e**) Obliterative phlebitis in IgG4-RD mastitis, x100, x400.

Figure 2 – IGM histopathology

(**a** and **b**) Epithelioid histiocytes, x400. (**c** and **d**) Well-formed granulomas and giant cells, x400.

Figure 3 – Quantification of IgG4 and IgG immunostaining

(**a**) Plot of the average number of IgG4+ plasma cells per high power field. The average IgG4/hpf was significantly higher for the IgG4-RD cases than the indeterminate cases ($P<0.01$). (**b**) Plot of the IgG4:IgG plasma cell ratio. The IgG4:IgG ratio is significantly greater in the IgG4-RD cases as compared to the IGM and indeterminate cases (each $P<0.001$). Solid lines represent means, dashed lines the diagnostic cutoffs.

Figure 4 – IgG4 and IgG immunostaining

Images of dual-stained (IgG4+ brown, IgG+ red) IgG4-RD mastitis cases (**a** and **b**) and IGM cases (**c** and **d**), x200.

Figure 1

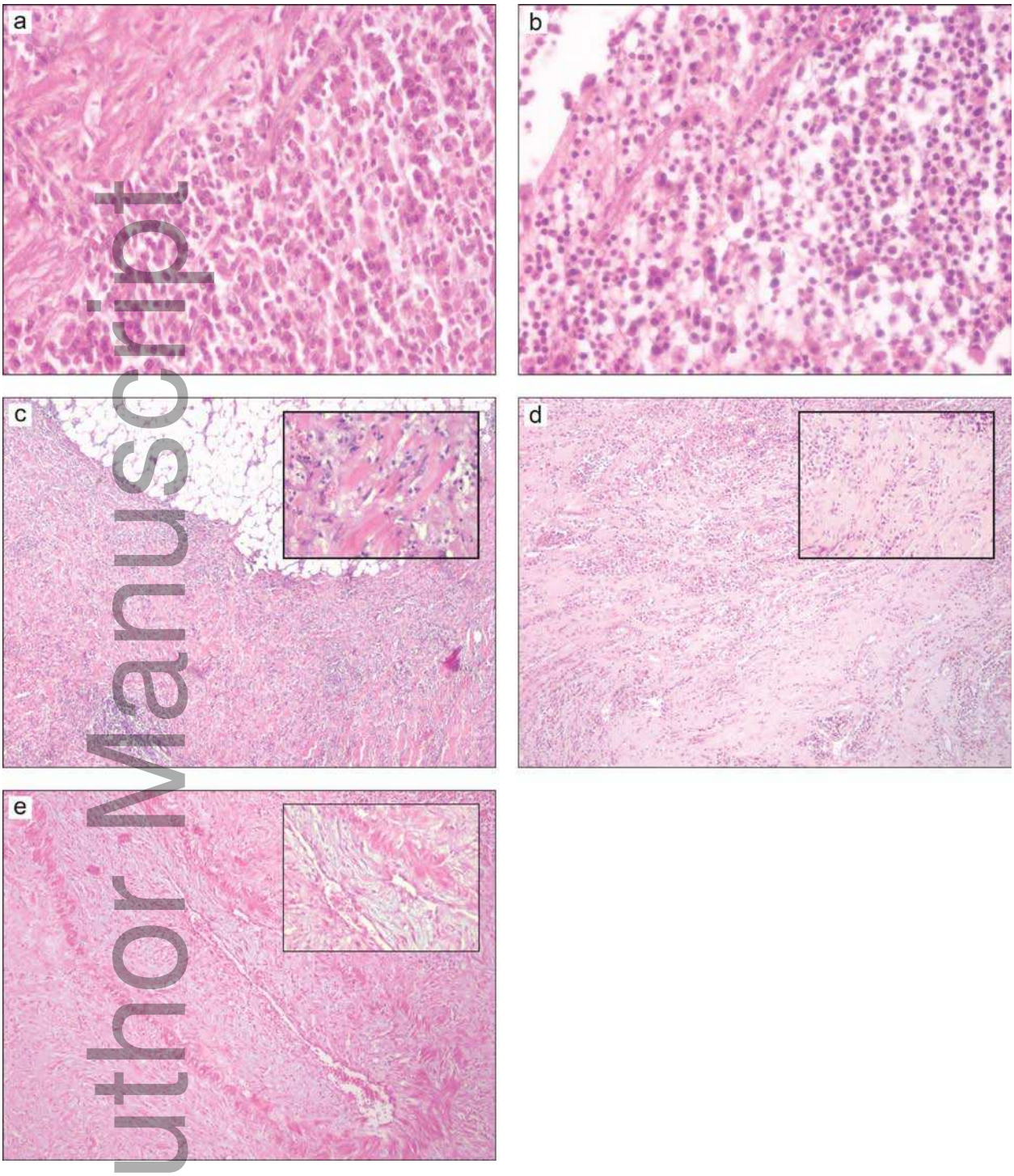

Figure 2

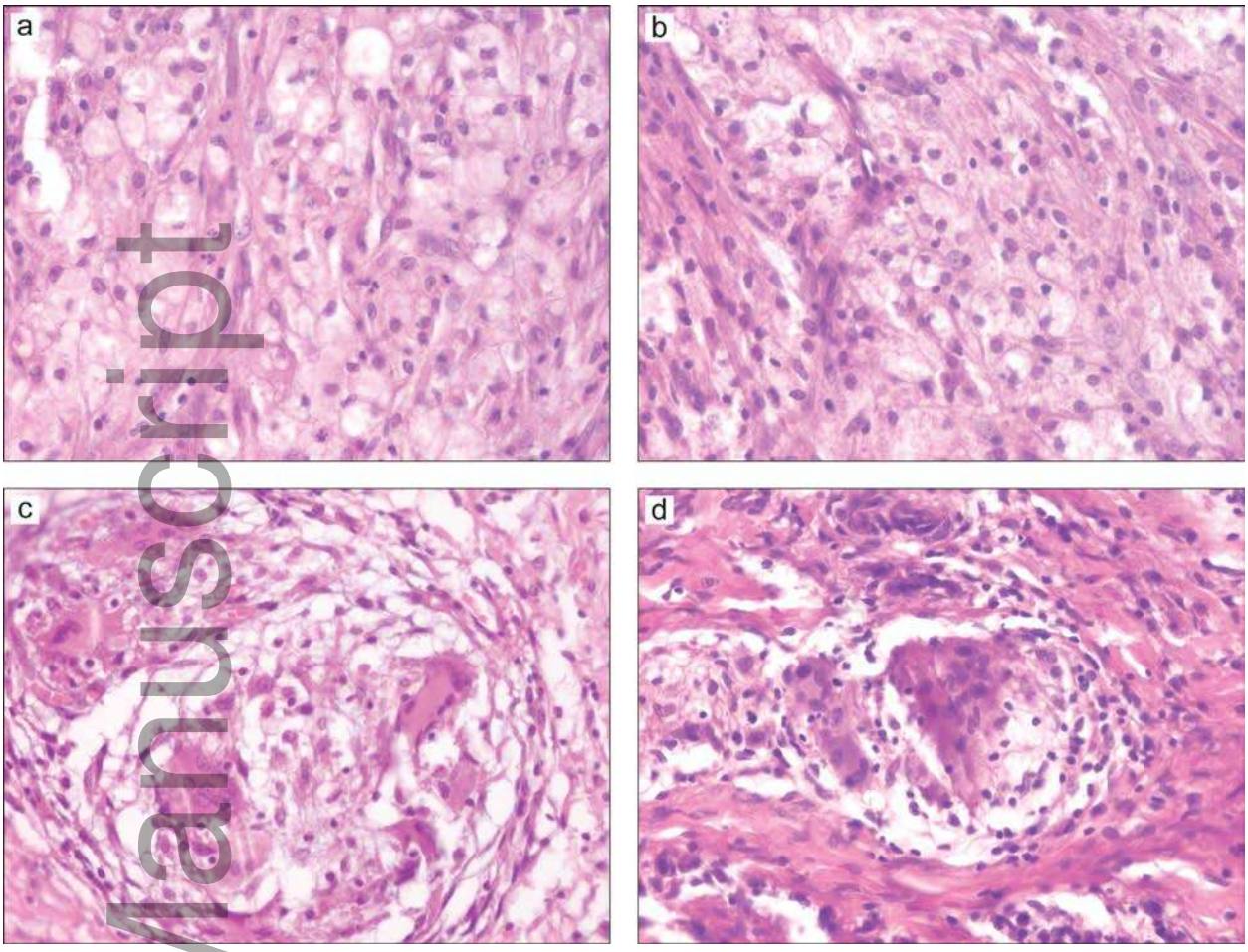

Figure 3

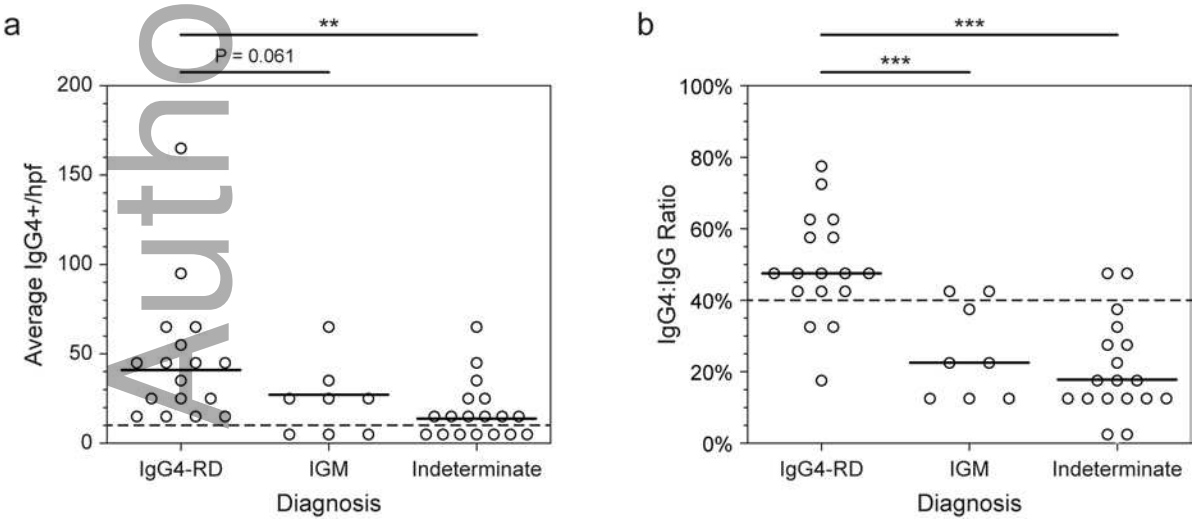

Figure 4