The alteration of lymphocyte subsets in idiopathic granulomatous mastitis

Abstract

Background and aim: This study analyzed peripheral blood lymphocyte subsets to determine their role in the etiopathogenesis of IGM.

Materials and methods: This study includes 51 pathologically proven IGM patients (active disease: 26 and in remission: 25) and 28 healthy volunteers. The analyses of lymphocyte subsets were performed by flow cytometric immunophenotyping.

Results: The percentage of T helper lymphocyte of all IGM patients were lower than control groups (p = .001). Absolute cytotoxic T lymphocyte count (p=.03), both percentage (p=.035) and absolute count (p=.002) of the natural killer cells, and both percentage (p=.038) and absolute count (p=.008) of natural killer T cells, were higher than the control group. The T helper lymphocyte percentage of the patients with active disease was lower than the control group (p = .0003). The absolute cytotoxic T lymphocyte (p=.029) and natural killer T cells (p=.012) of the patients with active disease were higher than the control group.

Conclusion: Idiopathic granulomatous mastitis is defined as a localized form of granulomatous disorders. However, the observed changes in T cells, NK and NKT cells suggest that there is systemic immune dysregulation in patients with IGM.

Keywords: Idiopathic granulomatous mastitis, lymphocyte subsets, autoimmunity, etiopathogenesis

1. INTRODUCTION

Idiopathic granulomatous mastitis (IGM), a benign inflammatory disease, is rarely seen worldwide. However, it is more frequent in some Middle Eastern countries such as Turkey, Egypt, and Iran and Asian countries such as China, India and Pakistan. Currently, no etiological factors accused, including autoimmune disorders, rheumatologic diseases, infectious causes and hormonal imbalances, could be proven. Granulomatous inflammation can be seen in various diseases of the breast, but the most common form is idiopathic in especially these countries [1-4]**.**

Remission and relapse of IGM in some patients with rheumatologic findings, such as arthritis and/or erythema nodule, suggest autoimmunity and immune dysregulation [5-11]. Erhan et al. [12] found T lymphocyte domination in the tissue of IGM patients. Similarly, in the study by Chen et al. [13], the predominance of CD3+ cells were detected higher than CD20+ cells in breast granulomatous tissue immunhistochemically. The high incidence of some human leukocyte antigens in patients with IGM also shed light on the etiopathogenesis [14].

Lymphocytes and their products constitute the most important part of the immune system. The main subgroups of lymphocytes are T-cells, B-cells, and natural killer (NK) cells. In order to establish and maintain a normal immune response, there must be a balance between the lymphocyte subgroups with regulatory and effector functions. There are almost no studies on autoimmunity considering it as an etiologic factor in IGM patients who may have abnormalities in humoral and cellular immune systems [15].

In this study, the changes of peripheral blood lymphocyte subsets in IGM patients and its possible role in pathogenesis were aimed to be investigated.

2. METHODS

2.1. Patients

Between March 2019 and May 2020, 51 patients with pathologically proven IGM and 28 healthy volunteers, as the control group, were enrolled. The IGM patients were divided into active IGM, and in remission IGM according to the activity of their disease. Active IGM (n: 26) was defined as patients with clinically and radiologically defined lesions with pathological diagnosis, whilst patients in remission (n: 25) had no clinical or radiological symptoms and findings except for their former pathological diagnosis. The criteria defined by Kessler and Wolloch [16] and detailed by Cohen [17] were used in the pathological diagnosis.

Demographic data including age, marital status, whether the birth, the number of children, breastfeeding history, and use of oral contraception, symptoms and signs were noted.

2.2.Flow cytometric analysis

The blood samples were collected during the first admission, before any treatment was administered in active IGM patients, whereas the samples were collected at least six months after the termination of treatment in patient with remission. In order to evaluate the lymphocyte subgroups, Using EDTA tubes, 2 ml blood samples were collected from patients and delivered to the pediatric immunology laboratory. Peripheral blood samples were collected in K2-EDTA anticoagulant. 100 µl anticoagulated whole blood was aliquoted into 5 ml polystyrene falcon flow cytometer tubes (Becton Dickinson, New Jersey, USA) and stained with Anti-Human CD3-PE-Cy5, CD4-PE, CD8-FITC, CD19-APC, CD16+CD56-PE, CD45-APC monoclonal antibodies (Biolegend, San Diego,

USA) at the manufacturer's recommended concentration. The tubes were incubated in the dark for 20 minutes at room temperature. Red blood cells were lysed by adding 2ml 1X red blood cells lysis buffer (Biolegend, San Diego, USA) and incubating in the dark at room temperature for 10 minutes. Then the tubes were centrifuged for five minutes at 1500 rpm. The cell pellet was washed once and re-suspended in 500 µl cell staining buffer (Biolegend, San Diego, USA). Lymphocyte subset analysis was performed using a FACS Aria III flow cytometer (Becton Dickinson, USA) and FACS Diva Version 6.1.3 Software (Becton Dickinson, New Jersey, USA) within 24 hours. Lymphocyte subsets were identified from lymphocyte population as Total T cells ($CD3^+$), T helper cells ($CD3^+CD4^+$), cytotoxic T cells ($CD3^+CD8^+$), B cells ($CD19^+$), Natural Killer cells (NK-$CD3^-CD16^+56^+$) and Natural Killer T cells (NKT-$CD3^+CD16^+56^+$).

2.3.Statistical analysis

SPSS-15 software was used for all statistical analyses. For numerical data, mean ± standard deviation (SD) or median (min-max) were used according to the distribution of data.

The Kruskall Wallis test was used to compare the ages of the groups. Student *T* test was used to compare the means of two independent groups. In comparing the means of more than two independent groups, one-way ANOVA was used. At one-way ANOVA, when a significant difference was found, Tukey post-hoc analysis was used for comparing groups.

In the comparison of categorical data, Fischer Exact test was used as the data did not meet the assumptions required for χ^2 test.

An alpha value (p) less than .05 was considered statistically significant.

3. RESULTS

Fifty-one IGM patients, and 28 healthy volunteers as the control group were enrolled. The median age of patient and control groups were 37 (26 to 64 years), and 34 years (20-52 years) respectively. There was no statistically significant difference in age between the patients and healthy volunteers (p = .31). Demographic and clinical features of all the patients enrolled and the treatment approaches are given in Table 1.

3.1.Comparison of control group and all patients' lymphocyte subsets

The absolute counts of leukocytes, neutrophils and monocytes in IGM patients were higher than in the control group. While lymphocyte percentage and T helper lymphocyte percentage were statistically lower in the patient group compared to the control, lymphocyte absolute count, T cytotoxic lymphocyte absolute count, natural killer cell percentage and absolute count and Natural Killer T cell percentage and absolute count were statistically higher in the patient group compared to the control group. T lymphocyte count and percentage, T helper lymphocyte absolute count, T cytotoxic lymphocyte percentage, total B lymphocyte percentage and absolute count, and double negative T cell percentage and absolute count were similar between the two groups. In addition, the CD4 / CD8 ratio was found to be statistically lower in the patient group compared to the control group. The absolute count and percentage of leukocytes, neutrophils, monocytes, lymphocytes, the lymphocyte subset values, and CD4/CD8 ratio were given in Table 2.

3.2.The comparisons of control group and the subgroups of patients

The absolute count and percentages of leukocytes, neutrophils, monocytes, and lymphocytes, the lymphocyte subset values of the control group and the subgroups of IGM patients were given in Table 3.

The leukocyte count and absolute counts of neutrophils, T cytotoxic lymphocytes, NK and NKT cells were higher in patients with active IGM disease than the control group. The percentages of neutrophils, T cytotoxic lymphocytes, NK and NKT cells were also statistically higher in patients with active IGM than the control group (Table 3).

The percentages of lymphocytes and T helper lymphocytes were statistically lower in patients with active disease than the control group.

CD4/CD8 ratio was lower in patients with active disease when compared with the control group.

4. DISCUSSION

Although some infectious causes and autoimmune processes, diabetes mellitus, and sarcoidosis can cause granulomatous reactions, the most common form of granulomatous inflammation in the breast is idiopathic [1]. In the present, the etiology of IGM is not clear. However, different factors including the deficiency of alpha-1 antitrypsin, oral contraceptives, smoking, hyperprolactinemia, ethnicity, autoimmunity, gestation, and birth and breast-feeding have been thought to be involved in the etiopathogenesis of IGM [1, 2]. Recently, autoimmunity and immune dysregulation have been emphasized, but studies on this subject are limited.

Some patients with IGM respond well to immunosuppressive drugs such as steroids and methotrexates; the presence of extramammary findings likes erythema nodosum or arthritis in some patients and some recent studies, although limited, suggest that more emphasis should be given to autoimmunity and immune dysregulation in IGM [3-13]. The aim of our study was to investigate the number of T and B lymphocytes and changes in NK and NKT cells in IGM patients.

In the study by Altıntoprak et al. [3], they investigated the autoantibodies including ANA and ENA levels in IGM. However, the authors could not demonstrate

any relationship between autoimmunity and IGM. In another important study, the authors serologically examined rheumatoid factor, ANA and anti-dsDNA in eight patients with IGM. Rheumatoid factor positivity was detected in six patients and ANA and anti-ds DNA positivity were detected in two patients. In these two patients, rheumatoid factor, ANA, and anti-ds DNA were all positive [18]. Unfortunately, in our article on autoantibodies in IGM, the newly online ahead of print, our findings did not support the clinical utility of autoantibodies including RF, ANA, anti-ds-DNA, pANCA and anti-CCP in IGM neither in diagnosis nor in follow up [19].

In a very important study by Erhan et al. [12], the authors investigated T and B lymphocyte markers in biopsy specimens in patients with IGM. In this study, T lymphocyte predominance was observed in biopsy specimens. Similarly, in the study by Chen et al. [13], the predominance of $CD3^+$ cells were detected higher than $CD20^+$ cells in breast granulomatous tissue immunhistochemically.

In our previous study, cytokine changes in patients with IGM were examined. The levels of interleukins -8, -10 and -17 in patients with IGM were found to be higher than the controls. We concluded that interleukins-8 and -17, proinflamatory cytokines, could have a role in the pathogenesis of IGM, but the low interleukin-10 levels suggested the reduction in the release of proinflamatory cytokines as well as suppressing their function. Therefore, it contributes to controlling the extent of disease [20].

Granulomatous inflammation, a form of delayed type hypersensitivity reaction, is a protective response to chronic infections with persistent pathogens such as mycobacterial infections. Granuloma formation may appear as a primary lesion in cases where the cause is unknown, as in sarcoidosis or IGM. In addition, the important role of

T helper cells in granuloma formation is well known [21]. Similar findings were found in the studies by Erhan et al. [12] and Chen et al. [13]. In our study, T helper cell ratio of all the IGM patients were lower than the control group. In subgroups analyses, T helper cell ratio of the patients with active disease was lower. Recruitment of T helper cells in granulomatous breast tissue may be a reason of low T cells in peripheral blood of the patients.

The role of T cytotoxic cells in some granuloma formation including listeriosis is known and T cytotoxic cells' accumulation are shown in effector phase of granuloma formation [22]. In this experimental study, listeriosis leading to rapid activation, proliferation and apoptosis of CD4 + and CD8 + T cells were revealed. In another study by Mannering and Cheers, chronic *Mycobacterium avium* infection was found to be associated with an increase in T-cell apoptosis and elevated, but sustained levels of *in vivo* proliferation [23]. In our study, absolute cytotoxic T cell count was found to be high in active IGM patients. This increase of T cytotoxic cells and the change of CD4/CD8 ratio may be related to a decrease in T helper cells. The limitation of our study was the lack of longitudinal follow-up of lymphocyte subsets in active patients.

Natural killer T cells play an important role in suppressing granuloma formation in the liver of mice by modulating the production of IFN-ɣ and IL-10. In this experimental study, they revealed increased granuloma formation in mice without NKT cells, which may be explained by an increased interferon gamma production. As a result, NKT cells were found to suppress granuloma formation [24]**.** We found that the NK cells and NKT cells were higher in IGM patients than the control group. The present results suggest that NKT cells may play a role in regulating and/or suppressing granuloma formation.

In conclusion, understanding the regulatory capacity of T cell subsets and NK cells in granuloma formation in IGM may allow understanding of the etiophatogenesis and lead to the development of new therapeutic agents for this disease which is full of secrets.

REFERENCES

1. Altintoprak F, Kivilcim T, Ozkan OV. Aetiology of idiopathic granulomatous mastitis. World Journal of Clinical Cases 2014; 2 (12): 852-858. doi:10.12998/wjcc.v2.i12.852
2. Benson JR, Dumitru D. Idiopathic granulomatous mastitis: presentation, investigation and management. Future Oncology 2016; 12 (11): 1381-1394. doi:10.2217/fon-2015-0038
3. Altintoprak F, Karakece E, Kivilcim T, Dikicier E, Cakmak G et al. Idiopathic granulomatous mastitis: an autoimmune disease? The Scientific World Journal 2013; 2013: 148727. doi: 10.1155/2013/148727
4. Sheybani F, Naderi HR, Gharib M, Sarvghad M, Mirfeizi Z. Idiopathic granulomatous mastitis: Long-discussed but yet-to-be-known. Autoimmunity 2016; 49 (4): 236-239. doi: 10.3109/08916934.2016.1138221
5. Salesi M, Karimifar M, Salimi F, Mahzouni P. A case of granulomatous mastitis with erythema nodosum and arthritis. Rheumatology International 2011; 31 (8): 1093-1095. doi: 10.1007/s00296-009-1273-0
6. Binesh F, Shiryazdi M, Bagher Owlia M, Azimi S. Idiopathic granulomatous mastitis, erythema nodosum and bilateral ankle arthritis in an Iranian woman. BMJ Case Reports 2013; 2013: bcr2012007636. doi: 10.1136/bcr-2012-007636

7. Alungal J, Abdulla MC, Narayan R. Idiopathic granulomatous mastitis with erythema nodosum and polyarthritis. Reumatismo 2016; 68 (2): 97-99. doi:10.4081/reumatismo.2016.844
8. Zabetian S, Friedman BJ, McHargue C. A case of idiopathic granulomatous mastitis associated with erythema nodosum, arthritis, and reactive cough. JAAD Case Reports 2016; 2 (2): 125-127. doi:10.1016/j.jdcr.2016.01.011
9. Akın M, Karabacak H, Esendağlı G, Yavuz A, Gültekin S et al. Coexistence of idiopathic granulomatous mastitis and erythemanodosum: successful treatment with corticosteroids. Turkish Journal of Medical Sciences 2017; 47 (5): 1590-1592. doi:10.3906/sag-1611-100
10. Fruchter R, Castilla C, Ng E, Pomeranz MK, Femia AN. Erythema nodosum in association with idiopathic granulomatous mastitis: a case series and review of the literature. Journal of the European Academy of Dermatology and Venereology 2017; 31 (9): e391-e393. doi: 10.1111/jdv.14194
11. Letourneux C, Diemunsch P, Korganow AS, Akladios CY, Bellocq JP et al. First report of granulomatous mastitis associated with Sjögren's syndrome. World Journal of Surgical Oncology 2013; 11: 268. doi:10.1186/1477-7819-11-268
12. Erhan Y, Veral A, Kara E, Ozdemir N, Kapkac M et al. A clinicopthologic study of a rare clinical entity mimicking breast carcinoma: idiopathic granulomatous mastitis. The Breast 2000; 9 (1): 52-56. doi: 10.1054/brst.1999.0072
13. Chen L, Zhang XY, Wang YV, Zhao QF, Ding HY. Granulomatous lobular mastitis: a clinicopathological analysis of 300 cases. Zhonghua Bing Li Xue Za Zhi. 2019; 48 (3): 231-236 (in Chinese). doi: 10.3760/cma.j.issn.0529-5807.2019.03.012

14. Koksal H. Human leukocyte antigens class I and II in patients with idiopathic granulomatous mastitis. American Journal of Surgery 2019; 218 (3): 605-608. doi: 10.1016/j.amjsurg.2019.01.038
15. Lee BW, Yap HK, Chew FT, Quah TC, Prabhakaran K et al. Age- and sex-related changes in lymphocyte subpopulations of healthy Asian subjects: from birth to adulthood. Cytometry 1996; 26 (1): 8-15. doi:10.1002/(SICI)1097-0320(19960315)26:1<8::AID-CYTO2>3.0.CO;2-E
16. Kessler E, Wolloch Y. Granulomatous mastitis: a lesion clinically simulating carcinoma. American Journal of Clinical Pathology 1972; 58: 642-646. doi:10.1093/ajcp/58.6.642
17. Cohen C. Granulomatous mastitis. A review of 5 cases. South African Medical Journal 1977; 52 (1): 14-16.
18. Ozel L, Unal A, Unal E, Kara M, Erdoğdu E et al. Granulomatous mastitis: is it an autoimmune disease? Diagnostic and therapeutic dilemmas. Surgery Today 2012; 42 (8): 729-733. doi: 10.1007/s00595-011-0046-z
19. Koksal H. The clinical utility of autoantibodies in patients with idiopathic granulomatous mastitis. Journal of Investigative Surgery. 2020 Dec 17: 1-5. doi: 10.1080/08941939.2020.1861666
20. Koksal H, Vatansev H, Artac H, Kadoglou N. The clinical value of interleukins-8, -10, and -17 in idiopathic granulomatous mastitis. Clinical Rheumatology 2020; 39 (5): 1671-1677. doi:10.1007/s10067-020-04925-8
21. Co DO, Hogan LH, Il-Kim S, Sandor M. T cell contributions to the different phases of granuloma formation. Immunology Letters 2004; 92 (1-2): 135-142. doi: 10.1016/j.imlet.2003.11.023

22. Mannering SI, Zhong J, Cheers C. T-cell activation, proliferation and apoptosis in primary Listeria monocytogenes infection. Immunology 2002; 106 (1): 87-95. doi: 10.1046/j.1365-2567.2002.01408.x

23. Mannering SI, Cheers C. Interleukin-2 and loss of immunity in experimental Mycobacterium avium infection. Infection and Immunity 2002; 70 (1): 27-35. doi: 10.1128/iai.70.1.27-35.2002

24. Kobayashi T, Kawamura H, Kanda Y, Matsumoto H, Saito S et al. Natural killer T cells suppress zymosan A-mediated granuloma formation in the liver by modulating interferon-γ and interleukin-10. Immunology 2012; 136 (1): 86-95. doi:10.1111/j.1365-2567.2012.03562.x

Table 1. The patients' clinical features

	With active disease (n=26)	In remission† (n= 25)	p-value
Parity status*			.999
Nulliparous	0 (0)	1 (4)	
Parous	25 (100)	24 (96)	
Breastfeeding history*	25 (100)	24 (96)	.999
Oral contraceptive use history	3 (12)	7 (28)	.15
Smoking*	2 (8)	3 (12)	.999
Menopausal status*			.49
Premenopausal	23 (92)	25 (100)	
Postmenopausal	2 (8)	0	
Symptom and signs			
Mass*	25 (100)	24 (96)	.66
Pain*	23 (92)	21 (84)	.999
Erythema	16 (64)	16 (64)	.999
Abscess	12 (48)	15 (60)	.39
Axillary lymphadenopathy	13 (52)	8 (32)	.25
Peau d'orange	2 (8)	2 (8)	.999
Nipple retraction*	0	4 (16)	.11
Ulcer*	3 (12)	2 (8)	.999
Fistula*	0	3 (12)	.11
Erythema nodosum*	1 (4)	3 (12)	.6

Treatment approaches	**		
Wait and watch		3 (12)	
Only antibiotics		4 (16)	
Drainage + antibiotics		9 (36)	
Intralesional steroid		5 (20)	
Systemic steroid		3 (12)	
Topical and intralesional steroid		1 (4)	

† For idiopathic granulomatous mastitis patients in remission clinical features at the time of first diagnosis are given. At the time of study these patients had no symptoms or findings.

*These categorical data applied to Fisher Exact test.

** Treatment approaches of active IGM patients were not given because the blood samples were collected during the first admission, before any treatment in these patients.

1 Table 2. The patients and control groups' lymphocyte subsets

	MAIN GROUPS		p-value
	All IGM patients (n=51)	Control groups (n=28)	
Leukocyte counts	8810.4 ± 2694.8	6824.2 ±1583.9	.001
Neutrophil			
%	61.46 ± 7.64	57.61± 5.08	.019
Absolute counts	5530.1 ± 2096.3	3946.3 ± 1020.3	< .0001
Monocyte			
%	5.91 ± 1.52	6.07 ± 1.51	.64
Absolute counts	530.7 ± 258.9	408.2 ± 116.4	.005
Lymphocyte			
%	27.85 ± 7.04	31.22 ± 5.22	.015
Absolute counts	2362.72 ± 702.99	2119.61 ± 531.82	.11
Lymphocyte subsets			
Total T lymphocyte			
%	74.22 ± 7.97	77.53 ± 5.56	.055
Absolute counts	1742.44 ± 513.46	1641.78 ± 420.49	.38
T helper lymphocyte			
%	42.87 ± 7.67	48.83 ± 5.56	.001
Absolute counts	1008.05 ± 343.49	1033.74 ± 275.30	.73
T cytotoxic lymphocyte			
%	25.08 ± 6.13	23.05 ± 5.23	.14
Absolute counts	585.51 ± 196.45	488.78 ± 175.81	.03

Natural killer cells			
%	13.9 ± 6.95	10.74 ± 4.7	.035
Absolute counts	332.88 ± 190.03	225.76 ± 104.5	.002
Total B lymphocyte			
%	9.64 ± 3.86	9.16 ± 3.75	.59
Absolute	228.85 ± 127.1	200.63 ± 110.96	.32
Natural killer T cell			
%	5.92 ± 3.41	4.53 ± 2.38	.038
Absolute	144.33 ± 111.11	94.17 ± 49.95	.008
Double negative T cells			
%	6.38 ± 3.48	5.64 ± 3.14	.35
Absolute	151.58 ± 91.59	119.34 ± 71.22	.11
CD4/CD8 ratio	1.85 ± 0.77	2.26 ± 0.74	.023

1 Table 3. The subgroups and control groups' hematological parameters and lymphocyte subsets

	SUBGROUPS			p-value
	With active disease (n=26)	In remission (n=25)	Control groups (n=28)	
Leukocyte counts	9545.0 ± 2214.8	8077.0 ± 2995.0	6824.2 ±1583.9	< .0001[a]
Neutrophil				
%	63.58 ± 7.68	5979.0 ± 6.69	57.61 ± 5.08	.005[b]
Absolute counts	6093.2 ± 1744.4	4920.25 ± 2304.4	3946.3 ± 1020.3	<.0001[c]
Monocyte				
%	5.59 ± 1.67	6.21 ± 1.33	6.07 ± 1.51	.277
Absolute counts	543.7 ± 229.5	516.6 ± 291.9	408.2 ± 116.4	.06
Lymphocyte				
%	26.5 ± 6.72	29.26 ± 7.22	31.22 ± 5.22	.02[d]
Absolute counts	2503.5 ± 800.7	2210.21 ± 556.16	2119.61 ± 531.82	.08
Lymphocyte subsets				
Total T lymphocyte				
%	74.03 ± 6.84	74.42 ± 9.15	77.53 ± 5.56	.15
Absolute counts	1833.62 ± 537.6	1643.67 ± 477.45	1641.78 ± 420.49	.25
T helper lymphocyte				
%	41.9 ± 5.85	43.89 ± 9.21	48.83 ± 5.56	.002[e]
Absolute counts	1047.13 ± 346.91	965.72 ± 342.0	1033.74 ± 275.30	.64
T cytotoxic lymphocyte				
%	25.57 ± 6.01	24.58 ± 6.34	23.05 ± 5.23	.28
Absolute counts	624.4 ± 210.78	543.37 ± 174.26	488.78 ± 175.81	.03[f]
Natural killer cells				
%	13.5 ± 6.54	14.27 ± 7.46	10.74 ± 4.7	.1
Absolute counts	347.72 ± 208.59	316.82 ± 170.66	225.76 ± 104.5	.02[g]
Total B lymphocyte				
%	9.47 ± 4.43	9.82 ± 3.25	9.16 ± 3.75	.82
Absolute	240.44 ± 154.84	216.31 ± 89.66	200.63 ± 110.96	.48
Natural killer T cell				
%	6.65 ± 3.36	5.16 ± 3.36	4.53 ± 2.38	.039[h]
Absolute	168.36 ± 104.73	118.3 ± 114.1	94.17 ± 49.95	.015[i]
Double negative T cells				
%	6.81 ± 3.7	5.92 ± 3.25	5.64 ± 3.14	.42

Absolute	167.85 ± 96.54	133.97 ± 84.38	119.34 ± 71.22	.1
CD4/CD8 ratio	1.74 ± 0.55	1.96 ± 0.94	2.26 ± 0.74	.045[j]

[a] With active disease & control group, $p < .0001$

[b] With active disease & control group, $p = .004$

[c] With active disease & control group, $p < .0001$

[d] With active disease & control group, $p = .033$;

[e] With active disease & control group, $p = .0003$; in remission & control groups, $p = .054$

[f] With active disease & control group, $p = .029$;

[g] With active disease & control group, $p = .023$

[h] With active disease & control group, $p = .034$

[i] With active disease & control groups, $p = .012$

[j] With active disease & control groups, $p = .036$