

Bone marrow findings in rheumatoid arthritis patients with peripheral cytopenias

Mohamed A. Abd El Hafez^a, Mohamed Helwa^b, Zeinab A.A. Kasemy^c

Departments of ^aInternal Medicine, ^bClinical Pathology, ^cPublic Health and Community Medicine (Epidemiology and Biostatistics), Faculty of Medicine, Menoufia University, Menoufia, Egypt

Correspondence to Mohamed A. Abd El Hafez, MD, 10 El-Shahed Ali Eleraqy Street, Houreen, Beriket Elsaba, Menoufia 32511, Egypt.
Tel: +20 106 619 0475; fax: +204822283012; e-mail: m_hafez79@yahoo.com

Received 12 September 2018

Accepted 7 November 2018

The Egyptian Journal of Internal Medicine
2019, 31:226–234

Objective

The aim was to study bone marrow (BM) cellularity, morphological characteristics, and types of cellular infiltrates in rheumatoid arthritis (RA) patients associated with peripheral cytopenias.

Background

RA is a systemic, autoimmune disease leading to joint destruction. BM is an important compartment in RA, where there are autoreactive lymphocytes, abnormal production of cytokines, and abnormal BM microenvironment, which may affect the integrity of the BM and accordingly may require specific modalities in treatment.

Patients and methods

In the current study, we examined 60 RA patients with cytopenias (defined according to WHO as hemoglobin level below 12 g/dl (woman), 13 g/dl (man), platelet count below $150 \times 10^9/l$, absolute neutrophil count below $1.8 \times 10^9/l$, and lymphocytes below $1.0 \times 10^9/l$). The diagnosis of RA was made according to the American College of Rheumatology (2010) criteria. All patients were subjected to thorough history taking, physical examination, imaging studies including abdominal ultrasound, and chest radiography and laboratory investigations including routine investigations, blood film, antinuclear antibody, antidouble stranded DNA antibody, rheumatoid factor, anticyclic citrullinated peptide (anti-CCP) antibody, and BM aspiration and biopsy. Flow cytometry immunophenotyping was performed to detect lymphocytic infiltration in the BM and its subtypes.

Results

The patients were predominantly women (91.7%); 40 (66.6%) patients had normocellular BM, 16 (26.6%) patients had hypercellular and four (6.6%) patients had hypocellular BM. Dysmyelopoiesis was present in five (8.3%) patients; dyserythropoietic and megaloblastoid changes were present in 26 (43.3%) patients whereas dysmegakaryopoiesis was present in six (10%) patients. BM eosinophils mean \pm SD was $2.95 \pm 1.51\%$. BM plasma cell mean \pm SD was 4.0 ± 2.35 . BM lymphocytes mean \pm SD was $15.65 \pm 6.75\%$ and was predominantly T-lymphocytes (mean \pm SD: $61.63 \pm 8.69\%$). BM-activated (CD25+) T-lymphocytes mean \pm SD was 23.8 ± 11.5 . Higher BM lymphocytes, T-cells, and plasma cell percentages were statistically associated with increased frequencies of positive anti-CCP, neutropenia, thrombocytopenia, and arthritis and were positively correlated to inflammatory markers (erythrocyte sedimentation rate, C-reactive protein, and ferritin). The percentage of activated (CD25+) BM T-lymphocytes was higher in RA patients with either positive C-reactive protein, positive anti-CCP, elevated ferritin, or pancytopenia. There was no significant relation between lymphocytic infiltration (either B-lymphocyte or T-lymphocyte) and type of BM cellularity.

Conclusion

Lymphocytic infiltration, especially T-lymphocytes is closely related to the activity markers in RA and to the type and degree of cytopenia in RA patients.

Keywords:

bone marrow, cytopenia, rheumatoid arthritis

Egypt J Intern Med 31:226–234

© 2019 The Egyptian Journal of Internal Medicine
1110-7782

Introduction

Rheumatoid arthritis (RA) is a systemic autoimmune disease characterized by chronic inflammation of the joint synovial tissue and subsequent destruction of the associated bone, cartilage, and soft tissues. T-cell-mediated immune response is considered as a critical contributor in RA initiation and progression. It has been hypothesized that autoreactive T-cells escaping

negative selection can recognize arthrogenic antigens and lead to autoimmunity and tissue destruction [1]. The pathogenic roles of circulating and tissue-localized

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

B-cells in RA can occur through several mechanisms that include autoantibody production, T-cell activation, and cytokine synthesis. Besides, the potential contribution of B-cells to T-cell differentiation and function, there is now growing evidence indicating that B-cells in RA can directly contribute to the local synthesis of a wide spectrum of cytokines acting on other pathologically relevant cell types [2]. The localization of inflammation to the synovium is primarily responsible for the dysregulated cellular and molecular mechanisms that ultimately lead to the typical features of RA, including joint pain, swelling, and structural changes. It is becoming increasingly apparent that a 'synoviocentric' model of RA could be limiting. Indeed, other anatomic compartments appear to be involved at all stages of the disease. The most important example comes from the recognition that clinical arthritis and subclinical synovitis are anticipated by a prearticular immunologic phase possibly developing in lymphoid tissues as well as in the lungs [3,4]. Full-blown joint disease itself spreads well beyond synovial tissue inflammation. Established arthritis can be characterized by the involvement of at least two other compartments that are in direct contact with the joint space. These include the draining lymph nodes [5] and, of special relevance, the subchondral bone marrow (BM) [6]. The aim of this work was to study the morphological characteristics of the BM and the types of cellular infiltrates in the BM in patients with RA associated with peripheral cytopenias.

Patients and methods

Sixty patients were selected from the outpatient and inpatient clinics of Rheumatology and Hematology Units, Menoufia University Hospital, in the period from January 2017 to January 2018. Informed consents were obtained from all patients in accordance with the local ethics committee. The diagnosis of RA was made according to the American College of Rheumatology (2010) [7]. Cytopenias were defined according to WHO as hemoglobin level below 12 g/dl (woman) and 13 g/dl (man), platelet count below $150 \times 10^9/l$, absolute neutrophil count below $1.8 \times 10^9/l$, and lymphocytes below $1.0 \times 10^9/l$ [8]. All patients were subjected to medical history taking and complete physical examination. Investigations were done for all patients including routine investigations (kidney function tests, liver function tests, fasting and postprandial blood glucose and complete blood count), blood film, iron profile, anti-HCV antibody, HBsAg, direct antiglobulin test, serology [rheumatoid factor (RF), antinuclear antibody (ANA), anti-dsDNA, antiphospholipid, and anticyclic citrullinated peptide

(anti-CCP) antibodies], and BM aspiration and biopsy. Abdominal ultrasound was performed to detect organomegaly (splenomegaly and hepatomegaly). Chest radiography was done to detect any suspected hilar lymphadenopathy or pulmonary fibrosis. Flow cytometric analysis of BM lymphocytes was studied. Patients aged less than 18 years old, patients known to have other causes of cytopenias (like thyroid disorders, chronic kidney disease, iron deficiency anemia, myelodysplastic syndrome), patients with HCV Ab, or HBsAg-positive patients, patients of hematological malignancies, or other collagen vascular disease were excluded from this study. RA patients without cytopenias were also excluded. A volume of 8 ml of blood was drawn through a venous access under strict aseptic conditions; 2 ml was delivered into an EDTA tube, mixed well, and were used to perform CBC (Sysmex XN-1000 hematology analyzer; Sysmex Corporation, Kobe, Japan), blood smears (to detect morphological features and exclude spurious cytopenias), and direct antiglobulin test. The remaining 6 ml was delivered into two plain tubes, left to clot then centrifuged, and sera were separated. Sera were used to perform the other investigations including liver functions, renal functions, blood sugar, iron profile, HCV Ab, and HBsAg (Cobas 6000 analyze series; Roche Diagnostics International Ltd, Rotkreuz, Switzerland). ANA test was done by immunofluorescence by incubating dilutions of patient sera with a monolayer of fixed, permeabilized cultured HEp-2 cells. Antibodies adherent to the cell monolayer were visualized with an antihuman immunoglobulin fluorescent reagent. The presence or absence of nuclear staining and the pattern of nuclear staining by fluorescence microscopy was identified. ELISA method was used to detect and measure anti-dsDNA antibody quantified in units/ml. RF was detected by its ability to attach to the Fc portion of IgG coating latex particles. A positive result is reported if agglutination occurs when equal volumes are mixed. Anti-CCP was performed on Cobas 6000 analyzer series (Roche Diagnostics International Ltd). BM aspiration was done from the posterior superior iliac crest under local anesthesia using lignocaine after taking the patient's consent using Salah and Klima aspiration needles to study the M/E ratio, morphology and adequacy of hematopoietic cells, the percentage of blasts, lymphocytes, histiocytes and plasma cells and BM iron. BM biopsy was done by Islam BM biopsy needles under local anesthesia to assess BM cellularity, infiltrations, bone trabeculae, abnormal localization of immature precursors, and BM fibrosis using reticulin stain. BM aspirate flow cytometry immunophenotyping was performed on BD FACS Calibur (BD Immunocytometry Systems, San Jose,

California, USA). Quantification of B was done using BD Pharmingen conjugated mouse monoclonal antihuman CD19, isotype IgG1, κ , catalog number 555413, while quantification of T was done using BD Pharmingen FITC-conjugated mouse monoclonal antihuman CD3, isotype IgG2a, κ , catalog number 555339. To study lymphocyte activation status BD Pharmingen, PerCP mouse IgG1, κ , antihuman CD25 immunoglobulin, catalog number 560503 was used. One hundred microliters of BM aspirates was added to a tube; then 5 μ l of each of the monoclonal antibodies was added. An isotypic control and an auto control tube were involved. The tubes were well and gently mixed and incubated for 15 min in the dark at room temperature (18°–25°C). Cells were subjected to red blood cell lysis by 2 ml of lysing solution for 3 min. Then the tubes were washed three times in PBS and finally the cells were suspended in 250 μ l of PBS for final flow cytometric analysis. Gating was done on lymphocyte population and the percentage of each subpopulation was measured.

Statistical analysis

Data were collected, tabulated, and statistically analyzed using an IBM personal computer with the statistical package for social sciences (SPSS) version 22 (SPSS Inc., Chicago, Illinois, USA). Quantitative data were presented in the form of mean, SD, and range. Qualitative data were presented in the form of numbers and percentages and analyzed by applying χ^2 and Fisher's exact tests. Independent sample *t* and analysis of variance tests were used for comparison between means of normally distributed quantitative variables. Mann-Whitney and Kruskal-Wallis tests were used for not normally distributed quantitative variables. Spearman's correlation was used to assess the strength and direction of correlation among nonparametric variables. A *P* value of less than 0.05 was considered statistically significant.

Results

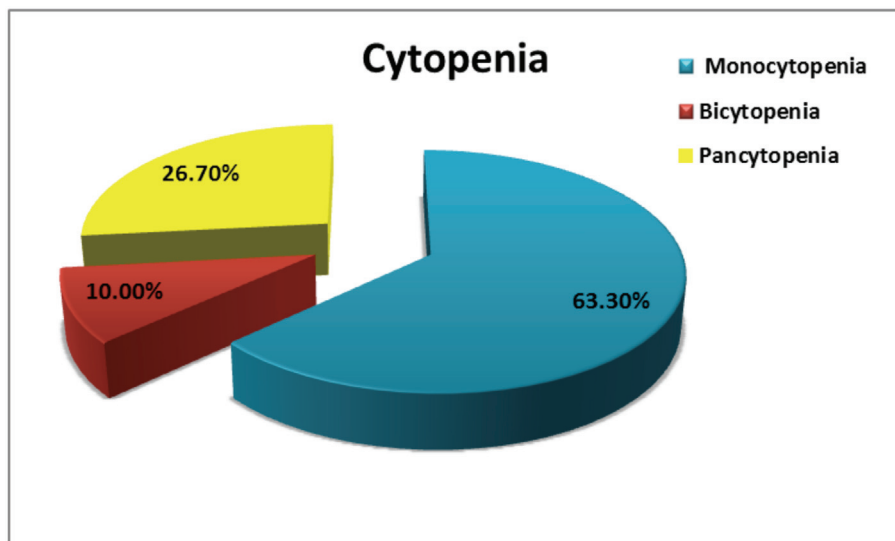
Descriptive demographic and clinical data of RA patients are detailed in Table 1. The mean age of RA patients was 40.01±9.05 years with a range of 24–55 years. They showed female predominance with women constituting 91.7%. Arthralgia and morning stiffness were the most frequent clinical symptoms (85 and 83.3%, respectively), while arthritis and splenomegaly were the most frequent clinical signs (66.7 and 50%, respectively) (Table 1). All patients were RF positive while anti-CCP was positive only in 34 (56.7%) patients. Erythrocyte sedimentation rate (ESR) was elevated in all patients with nine patients having an ESR higher than

Table 1 Descriptive data of demographic and clinical characteristics of the studied patients with rheumatoid arthritis

General characteristics	Rheumatoid arthritis patients (n=60) [n (%)]
Age (years)	
Mean±SD	40.01±9.05
Range	24–55
Sex	
Male	5 (8.3)
Female	55 (91.7)
Arthralgia	51 (85.0)
Arthritis	40 (66.7)
Morning stiffness	50 (83.3)
Fever	22 (36.7)
Pleural effusion	2 (3.3)
Pericardial effusion	1 (1.7)
Rheumatoid nodules	3 (5.0)
Palpable purpura	2 (3.3)
Splenomegaly	30 (50.0)
Lymphadenopathy	3 (5.0)

100 mm/first hour while the C-reactive protein (CRP) was positive in 49 (81.75%) patients with five patients having CRP levels more than 100 mg/l. Similarly, serum ferritin was elevated in 48 patients with 13 patients having elevated serum ferritin of more than 1000 ng/ml. ANA was positive in three patients. All patients were negative for anti-dsDNA and antiphospholipid antibodies (Table 2). Hemogram and BM findings are detailed in Table 3. Hemoglobin mean±SD was 8.56 ±1.12 g/dl while the mean corpuscular volume mean±SD was 86.90±7.59 fl. Platelet count mean±SD was 198.41 ±105.60 ×10³/ μ l while the total leukocytic count mean±SD was 6.09±2.53×10³/ μ l. Peripheral blood lymphocyte absolute count mean±SD was 2.03 ±0.75×10³/ μ l, while the neutrophil count mean±SD was 3.52±1.86×10³/ μ l. Reticulocytic percentage mean ±SD was 1.25±0.64%. All patients had anemia with isolated anemia seen in 38 (63.3%) patients, while 20 (33.3%) patients had thrombocytopenia and 18 (30%) patients had neutropenia. No patients had isolated thrombocytopenia or isolated neutropenia. Bicytopenia (anemia and thrombocytopenia) was present in four (6.7%) patients and bicytopenia (anemia and neutropenia) was present in two (3.3%) patients. Pancytopenia was present in 16 (26.7%) patients. All patients were anemic (Table 3), (Fig. 1). BM study has shown that 40 (66.7%) patients had normocellular BM, 16 (26.6%) patients had hypercellular, and four (6.7%) patients had hypocellular BM. M/E ratio was normal in 38 (63.3%) patients, decreased in 16 (26.6%) patients, and high in six (10%) patients. Dysplastic changes were seen in RA patients but dysplasia never affected 10% or more of lineage cells. Dysmyelopoiesis was present in five

Figure 1



Type of cytopenias in the studied patients with rheumatoid arthritis.

Table 2 Descriptive data of inflammatory markers in the studied patients with rheumatoid arthritis

Inflammatory markers	Rheumatoid arthritis patients (n=60) (%)
CRP (mg/l)	
Mean±SD	58.37±37.38
Range	1–130
<3	11
3–100	44
>100	5
ESR (mm/first hour)	
Mean±SD	74.60±26.16
Range	25–122
Elevated but <100	51
Elevated >100	9
Serum ferritin (ng/ml)	
Mean±SD	692.75±432.01
Range	80–2000
Normal	12
Elevated but <1000	35
Elevated but >1000	13
Positive CRP [n (%)]	49 (81.7)
Positive anti-CCP [n (%)]	34 (56.7)
Positive ANA [n (%)]	3 (5.0)

All patients had negative anti-dsDNA and antiphospholipid antibodies. ANA, antinuclear antibody; CCP, cyclic citrullinated peptide; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate.

(8.3%) patients; dyserythropoietic and megaloblastoid changes were present in 26 (43.3%) patients, while dysmegakaryopoiesis was present in six (10%) patients. BM eosinophils mean±SD was 2.95±1.51% and range 1–7%. BM plasma cell mean±SD was 4.0±2.35 and range 1–9. BM lymphocytes mean±SD was 15.65±6.75% and range 6–35%. Of these lymphocytes, T-lymphocytes

mean±SD was 61.63±8.69% and range 45–82% and B lymphocytes mean±SD was 35.66±8.57% with range 16–51%. BM-activated (CD25+) lymphocytes mean±SD was 23.8±11.5 and range 8–48 (Table 3). Higher BM lymphocytes, T-cells, and plasma cell percentages were statistically associated with increased frequencies of positive anti-CCP, neutropenia, thrombocytopenia, and arthritis and were positively correlated to inflammatory markers (ESR, CRP, and ferritin); however, B-cells had exactly the opposite associations. The percentage of BM eosinophils was higher in anti-CCP-negative RA patients and was negatively correlated to ESR. Neither of BM lymphocytes, T-cells, B-cells, plasma cells, or eosinophil percentages had associations with splenomegaly or BM cellularity (Table 4). The percentage of activated (CD25+) BM T-lymphocytes was higher in RA patients with positive CRP, positive anti-CCP, elevated ferritin, and pancytopenia (Table 5). Pancytopenia patients had statistically higher incidence of arthritis, positive anti-CCP, and inflammatory markers (hyperferritinemia, positive CRP) than monocytopenia patients. On the other hand, no significant difference was present between monocytopenia, bicytopenia, and pancytopenia patients as regards BM cellularity ($P=0.256$), BM dysplasia ($P=0.605$), or splenomegaly ($P=0.491$) (Table 6)

Discussion

Alongside its hematopoietic function and its role in the early selection of lymphocytes, the BM is an immune-regulatory organ involved in migration, selective retainment, and function of innate and adaptive

Table 3 Descriptive data of the hemogram and bone marrow findings of the studied patients with rheumatoid arthritis

	Rheumatoid arthritis patients (n=60)	
	Mean±SD	Range
Hemoglobin (g/dl)	8.56±1.12	4.80–10.80
MCV (fl)	86.90±7.59	75–100
Platelets (×10 ³ /μl)	198.41±105.60	35–345
Total leukocyte count (×10 ³ /μl)	6.09±2.53	2–10.50
Absolute neutrophil count (×10 ³ /μl)	3.52±1.86	0–7.10
Absolute lymphocyte count (×10 ³ /μl)	2.03±0.75	0–3.30
Reticulocytic percentage	1.25±0.64	0.40–3.80
Anemia [n (%)]	60	100
Isolated anemia [n (%)]	38	63.3
Thrombocytopenia [n (%)]	20	33.3
Leukopenia [n (%)]	18	30.0
Anemia and thrombocytopenia [n (%)]	4	6.6
Anemia and leukopenia [n (%)]	2	3.3
Pancytopenia [n (%)]	16	26.6
Bone cellularity [n (%)]		
Normocellular BM	40	66.7
Hypercellular BM	16	26.7
Hypocellular BM	4	6.6
M/E ratio [n (%)]		
Normal	38	63.3
Decreased	16	26.7
Increased	6	10.0
Dyserythropoiesis and megaloblastoid changes [n (%)]	26	43.3
Dysmegakaryopoiesis [n (%)]	6	10.0
Dysmyelopoiesis [n (%)]	5	8.3
Bone marrow eosinophil %	2.95±1.51	1–7
Bone marrow plasma cell %	4.0±2.35	1–9
Bone marrow lymphocyte %	15.65±6.75	6–35
Bone marrow T-cells %	61.63±8.69	45–82
Bone marrow B-cells %	35.66±8.57	16–51
Bone marrow activated (CD25 ⁺) T-lymphocytes %	23.8±11.5	8–48

BM, bone marrow; MCV, mean corpuscular volume.

immune cells [9]. A variety of morphological, immunophenotypic, and functional abnormalities in BM cells in RA have been detected as a consequence of augmented local production of inflammatory cytokines and cell–cell interactions. Both myeloid and lymphoid lineage cells appear affected at some stage of the disease [10]. Previous studies by Rudnicka *et al.* [11], Kuca-Warnawin *et al.* [12] and Kurowska and Kuca-Warnawin [13] showed the presence of cellular infiltrates that replace yellow adipose tissue in the BM of RA patients, described on magnetic resonance scans as BM edema. The cellular infiltrates found in RA BM consist of immunological cells that may form aggregates resembling germinal centers in secondary lymphoid organs. Flow cytometric analysis showed an increased number of mononuclear cells and accumulation of activated T-cells and B-cells in the BM of RA patients. Our results showed more that the BM T lymphocyte population was higher than the B-lymphocyte population (61.63±8.69 vs. 35.66±8.57%, respectively). We found lymphoid infiltrate in most

patients included in this study and this agreed with Tomita *et al.* [14] who found that the absolute number of mononuclear cells including lymphocytes was markedly increased in BM aspirates in RA patients; and agreed with Kuca-Warnawin *et al.* [12] and Engels *et al.* [15] who found expanded lymphoid aggregates in BM biopsy of RA patients. The lymphoid infiltrate was mainly T-lymphocytes and this may support the significant role of T-cells in RA pathogenesis; this agrees with Tomita *et al.* [14] and Doita *et al.* [16] who found abnormal accumulation of T-lymphocytes in the BM of RA patients. We found significant relation between BM lymphocytes, T-cells, and plasma cell percentages with increased frequencies of positive anti-CCP, neutropenia, thrombocytopenia, and higher levels of inflammatory markers (ESR, CRP, and ferritin). This may indicate a significant relation of lymphoid infiltrate in the BM with RA activity and may confirm the new consideration of the role of BM in RA as it is considered now as an important compartment in RA as mentioned by Kurowska and

Table 4 Association of the percentage of bone marrow lymphocytes, plasma cells, and eosinophils with clinical and laboratory findings in rheumatoid arthritis patients

	BM total lymphocyte% (mean±SD)	BM B-cells% (mean±SD)	BM T-cells% (mean±SD)	BM plasma cells% (mean±SD)	BM eosinophils% (mean±SD)
Anti-CCP (mean±SD)					
Positive	19.75±5.37	31.36±7.44	66.02±7.61	5.19±2.18	2.58±1.44
Negative	9.50±2.71	42.12±5.65	55.04±5.49	2.20±1.17	3.50±1.47
	<0.001*	<0.001*	<0.001*	<0.001* [#]	0.010* [#]
CRP	0.685	0.699	-0.674	0.698	-0.234
(correlation)	<0.001*	<0.001*	<0.001*	<0.001*	0.072
ESR	0.673	0.626	-0.606	0.651	-0.289
(correlation)	<0.001*	<0.001*	<0.001*	<0.001*	0.025*
Ferritin	0.693	0.615	-0.584	0.655	-0.234
(correlation)	<0.001*	<0.001*	<0.001*	<0.001*	0.072
Bone cellularity					
Normocellular BM	14.77±6.24	61.0±8.96	36.25±8.90	4.0±2.40	2.70±1.28
Hypercellular BM	15.62±6.56	61.06±7.98	36.25±7.76	3.68±2.24	3.62±1.85
Hypocellular BM	24.50±7.85	70.25±4.35	27.50±4.35	5.25±2.50	2.75±1.70
	0.091	0.121	0.144	0.500 ^{##}	0.204 ^{##}
Thrombocytopenia					
Yes	20.35±5.80	30.15±7.91	67.60±7.97	5.60±2.58	2.70±1.71
No	13.30±5.97	38.42±7.57	58.65±7.48	3.20±1.78	3.07±1.40
	<0.001* [#]	<0.001*	<0.001*	<0.001* [#]	0.134 [#]
Neutropenia					
Yes	21.77±4.69	28.16±6.82	69.27±7.29	5.94±2.12	2.83±1.75
No	13.02±5.74	38.88±7.16	58.35±7.07	3.16±1.93	3.0±1.41
	<0.001* [#]	<0.001*	<0.001*	<0.001* [#]	0.449
Arthritis					
Yes	18.85±5.84	32.02±7.77	62.27±7.95	4.90±2.27	2.75±1.59
No	9.25±2.65	42.95±4.55	54.35±4.61	2.20±1.23	3.35±1.26
	<0.001*	<0.001*	<0.001*	<0.001* [#]	0.058 [#]
Splenomegaly					
Yes	15.26±7.06	35.76±8.38	61.56±2.39	3.46±2.06	2.86±1.50
No	16.03±6.54	35.56±8.76	61.70±9.14	4.53±2.54	3.03±1.54
	0.667 [#]	0.929	0.953	0.105 [#]	0.720 [#]

BM, bone marrow; CCP, cyclic citrullinated peptide; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate. *Significant. [#]Mann-Whitney test. ^{##}Kruskal-Wallis test, the other statistical tests used included *t*, analysis of variance and Spearman's correlation.

Table 5 Association between the percentage of activated bone marrow lymphocytes and serum ferritin, anticyclic citrullinated peptide, C-reactive protein, and peripheral blood cytopenia

	Percentage of activated (CD25 positive) BM T-lymphocyte		
	Mean±SD (%)	Test of significance	P value
Serum ferritin			
Normal (N=12)	18.66±4.01	Mann-Whitney 4.27	<0.001*
Increased (N=48)	27.39±9.39		
Anti-CCP			
Positive (N=36)	29.75±8.37	<i>t</i> 10.2	<0.001*
Negative (N=24)	19.50±5.71		
CRP			
Positive (N=49)	27.22±12.73	Mann-Whitney 4.04	<0.001*
Negative (N=21)	16.63±5.11		
Peripheral blood cytopenia			
Monocytopenia (N=38)	12.92±5.86	Kruskal-Wallis 18.85	<0.001*
Bicytopenia (N=6)	16.16±5.45		
Pancytopenia (N=16)	28.93±8.89		

BM, bone marrow; CRP, C-reactive protein. *Significant.

Table 6 Association between peripheral blood cytopenia and bone marrow cellularity, bone marrow dysplasia, ferritin, splenomegaly, arthritis, and anticyclic citrullinated peptide

	Type of cytopenia [n (%)]			Tests of significance	P value
	Monocytopenia (anemia) (group 1) (N=38)	Bicytopenia (group 2) (N=6)	Pancytopenia (group 3) (N=16)		
Bone cellularity					
Normal cellularity	27 (71.1)	4 (66.7)	9 (56.3)	χ^2	0.256
Hypercellularity	10 (26.3)	2 (33.3)	4 (25.0)	5.32	
Hypocellularity	1 (2.6)	0 (0.0)	3 (18.8)		
BM dysplasia					
Absent (N=32)	23 (60.5)	3 (50.0)	6 (37.5)	χ^2	0.605
Unilineage (N=15)	8 (21.1)	2 (33.3)	5 (31.2)	2.72	
Multilineage (N=13)	7 (18.4)	1 (16.7)	5 (31.2)		
Splenomegaly					
Present	17 (44.7)	3 (50.0)	10 (62.5)	χ^2	$P_1=1.0 P_2=0.371 P_3=0.655$
Absent	21 (55.3)	3 (50.0)	6 (37.5)	1.42 $P=0.491$	
Arthritis					
Positive	19 (50.0)	5 (83.3)	16 (100.0)	χ^2	$P_1=0.197 P_2=0.0003^* P_3=0.272$
Negative	19 (50.0)	1 (16.7)	0 (0.0)	13.50 $P=0.001^*$	
Anti-CCP					
Positive	16 (42.1)	5 (83.3)	15 (93.8)	χ^2	$P_1=0.088 P_2=0.0006^* P_3=0.48$
Negative	22 (57.9)	1 (16.7)	1 (6.2)	14.02 $P<0.001^*$	
Ferritin					
Normal	11 (28.9)	1 (16.7)	0 (0.0)	χ^2	$P_1=1.0 P_2=0.022^* P_3=0.272$
Increased	27 (71.1)	5 (83.3)	16 (100.0)	5.94 $P=0.051$	
CRP					
Normal	9 (23.7)	2 (33.3)	0 (0.0)	5.22	$P_1=0.63 P_2=0.045^* P_3=0.064$
Increased	29 (76.3)	4 (67.7)	16 (100)	$P=0.072$	

BM, bone marrow; CCP, cyclic citrullinated peptide; CRP, C-reactive protein; P_1 , group 1 versus group 2; P_2 , group 1 versus group 3; P_3 , group 2 versus group 3. *Significant.

Kuca-Warnawin [13]. The same significant relation was present between lymphoid infiltrate and arthritis. This again may indicate a significant role of the BM as one of the important compartments in RA and its relation to synovitis. This agrees with McQueen and Ostendorf [17] and Bugatti *et al.* [18] who suggest that inflammation in BM arises independently of the pathologic processes operating 'inside the joint,' and represents an early immunopathological event in RA. Moreover, Bugatti *et al.* [18] mentioned that data from experimental animal studies showed the enlargement of canals in the cortical bone that connects BM to the synovium in the preclinical phase of RA. This phenomenon may facilitate migration of precursor cells of synoviocytes and osteoclasts from BM directly to the synovial membrane to excite an inflammatory response and destruction processes. Activated T-lymphocytes were found in the BM of our patients. This agrees with Kuca-Warnawin *et al.* [12] The percentage of activated (CD25⁺) BM T-lymphocytes was higher in RA patients with each of positive CRP, positive anti-CCP, elevated ferritin, and pancytopenia. This may support the significant role

of T-cells in RA pathogenesis. Our results showed that 30 patients had splenomegaly (50%) and this is in agreement with Nishiya *et al.* [19], who showed that 52% (26/50) had splenomegaly. Nishiya *et al.* [19] also noticed increased splenomegaly in patients with leukopenia, but there was no significant difference as regards hemoglobin concentrations or platelet cell counts. This disagrees with our results that showed no significant difference between the presence of splenomegaly and type of cytopenia. In our study, splenomegaly was found in 44.7% of patients with anemia and 50% in patients with bicytopenia and 62.5% of patients with pancytopenia. Although it was insignificant, splenomegaly was present more in RA patients with pancytopenia. Spleen may be enlarged to remove immune complexes and the resultant pancytopenia may be due to hypersplenism. The present study showed that 40 (66.7%) patients had arthritis. Arthritis was present in 50% of patients who presented with anemia, 83.3% of patients with bicytopenia (anemia with thrombocytopenia or leukopenia), and in 100% of patients with

pancytopenia. So, there is a significant relation between presence of arthritis and severity of cytopenia, so the presence of arthritis as an early articular manifestation of RA can predict the severity of cytopenia as one of the important extra-articular manifestations of RA. Rosenthal and Farhi [20] studied 35 patients and showed increased plasma cells in the BM of RA patients. Yetgin *et al.* [21] studied 17 patients with juvenile RA and found marked dysplasia in myeloid, erythroid, and megakaryocytic series with no correlation with disease activity. Hunt *et al.* [22] examined 38 RA patients and found mainly lymphoid aggregates, megaloblastoid erythroid lineage, and features of dysplasia especially abnormal localization of immature precursors. In our study, dysmyelopoiesis was present in 8.3% patients while dyserythropoiesis and megaloblastoid changes were reported in 43.3% patients and dysmegakaryopoiesis was present in 10% patients. BM plasma cell infiltration and lymphocytic infiltration ranges were 1–9% and 6–35%, respectively. In our study, we did not find any significant relation between the type and number of cytopenia and BM cellularity. We had 38 patients with isolated anemia, 27/38 patients (71.1%) had normocellular marrow, 10/38 patients (26.3%) had hypercellular marrow, and 1/38 patients (2.6%) had hypocellular marrow. All the cases with normocellular marrow had increased serum ferritin; so, the cause of anemia is mostly anemia of chronic disease associated with active RA. The remaining 11 cases had 10 patients with hypercellular marrow with normal serum ferritin. This may refer to a state of ineffective hematopoiesis or acquired dysplasia associated with active RA, the remaining one patient with anemia had BM suggesting immune-mediated pure red cell aplasia which can accompany RA and is immune mediated. We had 6/38 patients with bicytopenia, four of them had anemia and thrombocytopenia and two patients had anemia and neutropenia, BM cellularity in six patients was normocellular (in four patients) and hypercellular (in two patients); so these patients mostly had immune thrombocytopenia and immune neutropenia accompanying RA. The anemia is mostly anemia of chronic disease as five of them had increased serum ferritin. The last group was patients with pancytopenia, they were 16/38 patients. All of them had increased serum ferritin and so again the degree of cytopenia may reflect disease activity; three of them (18.8%) had hypocellular marrow (aplastic anemia), 9/16 patients (56.3%) had normocellular marrow, and 4/16 (25.0%) patients had hypercellular marrow. Our results showed that 34 (56.7%) patients had positive anti-CCP. This is nearly in agreement with Kapitány *et al.* [23], Bizzaro *et al.* [24], Bas *et al.* [25], Zeng *et al.* [26], and Ronnelid *et al.* [27] who showed positive anti-CCP

in 62, 44, 56, 47, and 57%, respectively. Our results showed that anti-CCP positivity is more with the increasing number of cytopenia as 42.1% of patients with anemia, 83.3% of patients with bicytopenia and 93.8% of patients with pancytopenia had positive anti-CCP. Anti-CCP usually predicts severe progressive disease, so the number and degree of cytopenia may predict the progressive disease. This also may add to our understanding of the etiopathogenesis of the different types of cytopenia in RA, which is directly related to the disease activity and may be related to BM suppression by inhibitory cytokines rather than hypersplenism or peripheral destruction. All the patients included in this study had anemia, and we found the increase of lymphocytic infiltration with the increased number of cytopenias to be more evident in patients with pancytopenia. Some of these patients had normocellular, hypercellular, or hypocellular marrow. Some had dysplastic features but the exact relation between lymphocytic infiltration and pancytopenia is not clear. Moreover, there was significant relation between lymphocytic infiltration and thrombocytopenia and neutropenia. T-lymphocytic infiltrate was more prominent than B-lymphocytic infiltrate, which confirms the more important role of T-lymphocyte in the pathogenesis of RA.

Conclusion and recommendations

Lymphocytic infiltration especially T-lymphocytes is closely related to the activity markers and to the type and degree of cytopenia in RA patients. We recommend BM aspiration and biopsy in patients with RA with unexplained cytopenia or if cytopenia persists after adequate treatment. We also recommend further studies on BM microenvironment in RA patients to unravel its role in disease pathogenesis and progression.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Wang D, Li Y, Liu Y, Shi G. The role of autoreactive T cell in the pathogenesis of rheumatoid arthritis and implications for T cell targeted vaccine therapy. *Minerva Med* 2015; 106:157–167.
2. Bugatti S, Vitolo B, Caporali R, Montecucco C, Manzo A. B cells in rheumatoid arthritis: from pathogenic players to disease biomarkers. *Biomed Res Int* 2014; 2014:681678.
3. Klareskog L, Rönnelid J, Lundberg K, Padyukov L, Alfredsson L. Immunity to citrullinated proteins in rheumatoid arthritis. *Annu Rev Immunol* 2008; 26:651–675.
4. Van de Sande MG, de Hair MJ, van der Leij C, Klarenbeek PL, Bos WH, Smith MD, *et al.* Different stages of rheumatoid arthritis: features of the synovium in the preclinical phase. *Ann Rheum Dis* 2011; 70:772–777.

- 5 Manzo A, Bombardieri M, Humby F, Pitzalis C. Secondary and ectopic lymphoid tissue responses in rheumatoid arthritis: from inflammation to autoimmunity and tissue damage/remodeling. *Immunol Rev* 2010; 233:267–285.
- 6 Schett G, Firestein GS. Mr Outside and Mr Inside: classic and alternative views on the pathogenesis of rheumatoid arthritis. *Ann Rheum Dis* 2010; 69:787–789.
- 7 Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham CO 3rd, *et al.* Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Arthritis Rheum* 2010; 62:2569–2581.
- 8 Valent P. Low blood counts: immune mediated, idiopathic, or myelodysplasia. *Hematology Am Soc Hematol Educ Program* 2012; 8:485–491.
- 9 Woodland DL, Blackman MA. Immunity: it's in our bones. *Immunity* 2005; 22:143–144.
- 10 Bugatti S, Manzo A, Caporali R, Montecucco C. Inflammatory lesions in the bone marrow of rheumatoid arthritis patients: a morphological perspective. *Arthritis Res Ther* 2012; 14:229.
- 11 Rudnicka W, Burakowski T, Warnawin E, Jastrzebska M, Bik M, Kontry E, *et al.* Functional TLR9 modulates bone marrow B cells from rheumatoid arthritis patients. *Eur J Immunol* 2009; 39:1211–1220.
- 12 Kuca-Warnawin E, Burakowski T, Kurowska W, Prochorec-Sobieszek M, Radzikowska A, Chorazy-Massalska M, *et al.* Elevated number of recently activated T cells in bone marrow of patients with rheumatoid arthritis: a role for interleukin 15? *Ann Rheum Dis* 2011; 70:227–233.
- 13 Kurowska W, Kuca-Warnawin E. New evidence for a role of bone marrow in the pathogenesis of rheumatoid arthritis. *Reumatologia* 2016; 54:215–216.
- 14 Tomita T, Kashiwagi N, Shimaoka Y, Ikawa T, Tanabe M, Nakagawa S, *et al.* Phenotypic characteristics of bone marrow cells in patients with rheumatoid arthritis. *J Rheumatol* 1994; 21:1608–1614.
- 15 Engels K, Oeschger S, Hansmann ML, Hillebrand M, Kriener S. Bone marrow trephines containing lymphoid aggregates from patients with rheumatoid and other autoimmune disorders frequently show clonal B-cell infiltrates. *Hum Pathol* 2007; 38:1402–1411.
- 16 Doita M, Maeda S, Kawai K, Hirohata K, Sugiyama T. Analysis of lymphocyte subsets of bone marrow in patients with rheumatoid arthritis by two colour immunofluorescence and flow cytometry. *Ann Rheum Dis* 1990; 49:168–171.
- 17 McQueen FM, Ostendorf B. What is MRI bone oedema in rheumatoid arthritis and why does it matter? *Arthritis Res Ther* 2006; 8:222.
- 18 Bugatti S, Caporali R, Manzo A, Vitolo B, Pitzalis C, Montecucco C. Involvement of subchondral bone marrow in rheumatoid arthritis: lymphoid neogenesis and in situ relationship to subchondral bone marrow osteoclast recruitment *Arthritis Rheum* 2005; 52:3448–3459.
- 19 Nishiya K, Hisakawa N, Hosokawa T, Hashimoto K. Enlarged spleen detected by abdominal ultrasonography in patients with RA. *Ann Rheum Dis* 2000; 59:750–752.
- 20 Rosenthal NS, Farhi DC. Bone marrow findings in connective tissue disease. *Am J Clin Pathol* 1989; 92:650–654.
- 21 Yetgin S, Ozen S, Saatci U, Bakkaloglu A, Besbas N, Kirel B. Myelodysplastic features in juvenile rheumatoid arthritis. *Am J Hematol* 1997; 54:166–169.
- 22 Hunt EK, Salama ME, Sever CE, Foucar K. Bone marrow examination for unexplained cytopenias reveals nonspecific findings in patients with collagen vascular disease. *Arch Pathol Lab Med* 2013; 137:948–954.
- 23 Kapitány A, Szabó Z, Lakos G, Aleksza M, Végvári A, Soós L, *et al.* Associations between serum anti-CCP antibody, rheumatoid factor levels and HLA-DR4 expression in Hungarian patients with rheumatoid arthritis. *Isr Med Assoc J* 2008; 10:32–36.
- 24 Bizzaro N, Mazzanti G, Tonutti E, Villalta D, Tozzoli R. Diagnostic accuracy of the anti-citrulline antibody assay for rheumatoid arthritis. *Clin Chem* 2001; 47:1089–1093.
- 25 Bas S, Genevay S, Meyer O, Gabay C. Anti-cyclic citrullinated peptide antibodies, IgM and IgA rheumatoid factors in the diagnosis and prognosis of rheumatoid arthritis. *Rheumatology (Oxford)* 2003; 42:677–680.
- 26 Zeng X, Ai M, Tian X, Gan X, Shi Y, Song Q, Tang F. Diagnostic value of anti-cyclic citrullinated Peptide antibody in patients with rheumatoid arthritis. *J Rheumatol* 2003; 30:1451–1455.
- 27 Ronnelid J, Wick M, Lampa J, Lindblad S, Nordmark B, Klareskog L, van Vollenhoven RF. Longitudinal analysis of citrullinated protein/peptide antibodies (anti-CP) during 5 year follow up in early rheumatoid arthritis: anti-CP status predicts worse disease activity and greater radiological progression. *Ann Rheum Dis* 2005; 64:1744–1749.