Studying of B-cell differentiation and macrophage colony-stimulating factors as new players in the diagnosis of rheumatoid arthritis

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Background/aim
There is an imperative, yet unmet need for the development of a biomarker panel for progression of rheumatoid arthritis (RA). This study aimed to investigate the reliability of B-cell differentiation factor (BCDF) and macrophage colony-stimulating factor (MCSF) as diagnostic and prognostic biomarkers of RA.
Patients and methods
A total of 25 RA patients were enrolled and divided into three subgroups according to the disease activity score, remission/minimal, moderate, and severe, in addition to 20 healthy controls. Enzyme-linked immunosorbent assay was used to measure serum BCDF, MCSF, and interleukin (IL)-2, and the calorimetric method was used to measure immunoglobulin G (IgG). In addition, the erythrocyte sedimentation rate was measured at 1 h in the RA patients.
Results
BCDF, MCSF, and IgG were significantly higher in the RA patients compared with the controls. BCDF was significantly higher in the severe disease group compared with the remission/mild and the moderate disease groups, whereas IgG was significantly higher among the moderate disease group compared with the remission/mild disease group, and among the severe disease group compared with the remission/mild disease group, whereas the erythrocyte sedimentation rate did not show any significant difference between the three subgroups of the disease group. BCDF delineates RA from controls at a cut-off value of 102 pg/ml, whereas at a cut-off value of 37.5 ng/l MCSF delineates RA from controls.
Conclusion
Serum BCDF and MCSF can be used as RA diagnostic biomarkers, whereas BCDF can be used additionally as a prognostic biomarker.

Keywords:
B-cell differentiation factor, interlukin-2, macrophage colony-stimulating factor, rheumatoid arthritis

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Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune disease that affects connective tissue of the synovial joints [1]. The disease mechanisms remain unclear, but are triggered by inflammatory mediators and infections [2]. Upon stimulation with the appropriate antigen and in the presence of a variety of lymphokines and monokines produced by T cells and monocytes, B cells begin to proliferate and differentiate into antibody-producing cells [3]. Al-Balaghi *et al.* [4], in their early study in 1984, concluded that synovial fluid in RA contains a B-cell differentiating factor (BCDF) as they found that RA is characterized by B-cell system hyperactivity, increased synthetic rate of DNA, non-T, nonmonocytic lympho- cytes as well as increased number of antibody-secreting cells both in the blood and in the synovial fluid.

Confirmed additional critical roles for B cells and their immunoglobulin products in the self-sustaining chronic inflammatory process led to the development of anti B cells-targeted therapies [5] and drugs attenuating the function of secreted and membrane-associated factors that contribute toward B-cell accumulation and survival at sites of disease [6]. B lymphocytes play several critical roles in the pathogenesis of RA as they are the source anticitrullinated protein antibodies (anticyclic citrullinated peptide) and the rheumatoid factor (RF) and contribute toward the activation of complement and the formation of immune complex in the joints. B cells are very efficient antigen-presenting cells, and can contribute toward T-cell activation through expression of costimulatory molecules; they respond and produce the cytokines and chemokines that

promote infiltration of leukocytes into the joints [7]. Macrophage activation and major histocompatibility complex class II expression are prominent features in RA joints. Granulocyte macrophage colony-stimulating factor (GM-CSF) is produced spontaneously by RA synovial cells in the absence of extrinsic stimuli [8] and is found abundantly in RA joints, where their blockage may reduce the inflammatory damage [9].

Molecular events that drive joint damage may precede disease onset, causing detrimental long-term effects such as disability [10,11]. Indeed, no single biomarker has emerged as a reliable predictor of damage in RA [12,13]; thus, there is great interest in identifying many biomarkers that could be incorporated into a signature predictive of disease progression to guide therapeutic decisions [14] and reserve aggressive treatment for patients prone to more severe forms of the disease [12]. Thus, we aimed, in our study, to test a panel of serological biomarkers comprising BCDF and macrophage colony-stimulating factor (MCSF) in addition to immunoglobulin G (IgG) and interleukin (IL)-2 from both the diagnostic and the prognostic aspects.

Patients and methods

Study design and patients' characteristics

A total of 25 RA patients were followed up at the rheumatology outpatient clinic of the center of excellence at National Research Centre, Cairo, Egypt. All patients provided their informed consent before their inclusion in the study. Patients who fulfilled the 1987 American College of Rheumatology classification revised criteria for RA [15] were recruited and screened.

Their mean age was 43.92±2.09 years; there were 23 women and two men. All patients were older than 18 years of age at disease onset. Patients were receiving steroid at a dose less than or equal to 7.5 mg, methotrexate at a dose ranging from 12.5 mg to 20 mg/week and/or leflunomide 20 mg/day and/or hydorxyclhoroquine 400 mg/day; none of the patients was or had been on biologic therapy. Patients with additional autoimmune disease other than RA, any concomitant systemic disease (hepatic, renal cardiological, etc.), or malignancy of any nature were excluded. All patients were subjected to a detailed assessment of history, and a thorough clinical examination, with a special focus on musculoskeletal examination with determination of tender joint count and swollen joint count. Disease activity was assessed using disease activity score-28 (DAS-28) [16]; accordingly, patients were divided into three groups: remission/minimal DAS-28 less than 3, moderate DAS-28 greater than or equal to 3–5, severe DAS-28 greater than 5. A total of 20 age-matched and sex-matched healthy volunteers were enrolled as a control group. All patients and controls were subjected to serum analysis for BCDF, MCSF, IL-2, and IgG.

Blood sampling

Blood samples were withdrawn from all participants and divided into two portions: one portion was allowed to clot and then centrifuged at 3000 rpm for 10 min; then, sera were isolated and stored at −80°C until the determination of laboratory investigations. The second portion was added to a sodium citrate (3.8%) tube to determine the erythrocyte sedimentation rate (ESR) at 1 h.

Biochemical assays

Serum levels of BCDF, M-CSF, IL-2, and IgG were measured according to the method described by the manufacturer using a commercial enzyme-linked immunosorbent assay kit produced by Glory Science Co. Ltd, China, whereas ESR was measured according to the method of Westergren [17].

Ethical consideration

Informed consent was obtained from all participants and the study protocol was approved by the ethical committee of the National Research Centre, and the study was carried out in accordance with the declaration of Helsinki 1964.

Statistical analysis

The study analyses were carried out using the statistical package for the social sciences, version 16 (statistical software; IBM, Chicago, Illinois, USA). The statistical significance was set at a P value of less than 0.05. All values are expressed as mean±SE and the differences between the two groups were calculated using Student's t-test. For the evaluation of the diagnostic performance of BCDF, MCSF, IL-2, and IgG, we used a receiver operating characteristic curve (ROC). The curve was constructed to show their sensitivity and specificity at different decision cut-off levels. In this type of curve, the x-axis represents the false-positive rate (1-specificity). The y-axis represents the true-positive rate (sensitivity). The best cut-off is the nearest point to the upper left corner. Area under the curve was constructed to determine the overall performance of the test. Sensitivity, specificity, and ROC analyses were carried out according to Sox *et al.* [18].

Results

Demographic and laboratory results of patients and controls

Demographic and laboratory characteristics of the patients and controls are shown in Table 1, which shows significantly higher levels of BCDF, MCSF, and IgG among the patients than the controls ($P<0.05$), whereas no statistically significant difference could be found between both groups in IL-2 ($P>0.05$).

Demographic and laboratory results of rheumatoid arthritis patients according to the disease activity

We further divided the patients into three subgroups: remission/mild, moderate, and severe according to the disease activity on the basis of DAS-28 [16], as shown in Table 2. The three groups did not show any significant difference in age or disease duration. On comparing the three subgroups in terms of BCDF, we found it to be significantly higher in the severe subgroup than the mild/remission and moderate subgroups. Also, a significantly higher IgG level was found in the severe subgroup compared with the mild/remission and the moderate subgroups together with a significantly higher level in the moderate subgroup compared with the mild/remission subgroup. There was no significant difference between the three groups in the MCSF.

Table 1 Demographic and laboratory results of the rheumatoid arthritis patients and control groups

Parameters	Controls (*N*=20) (17 women, three men)	RA patients (*N*=25) (23 women, two men)
Age (years)	38.45±1.91	43.92±2.09
BCDF (pg/ml)	102±9.43	223±21.28**
MCSF (ng/l)	75.57±21.1	149±23.43*
IL-2 (pg/ml)	121±41.11	55.08±6.73
IgG (mg/dl)	151±7.78	222±4.00**

All data are expressed as mean±SE; BCDF, B-cell differentiation factor; IgG, immunoglobulin G; IL-2, interlukin-2; MCSF, macrophage colony-stimulating factor; *Significant difference from the control group at $P<0.05$; **Highly significant difference from the control group at $P<0.01$.

Diagnostic accuracy of B-cell differentiation factor, macrophage colony-stimulating factor, and immunoglobulin G

As mean levels of BCDF, MCSF, and IgG showed significant differences between RA patients and controls, an ROC curve was constructed to assess their diagnostic accuracy. The IgG showed an accuracy of 0.955 at a cut-off value of 195 with a sensitivity of 0.88 (Fig. 1). The BCDF showed an accuracy of 0.925 at a cut-off value of 102 with a sensitivity of 0.800 (Fig. 2). The MCSF showed an accuracy of was 0.741 at a cut-off value of 37.5 with a sensitivity of 0.60 (Fig. 3).

Discussion

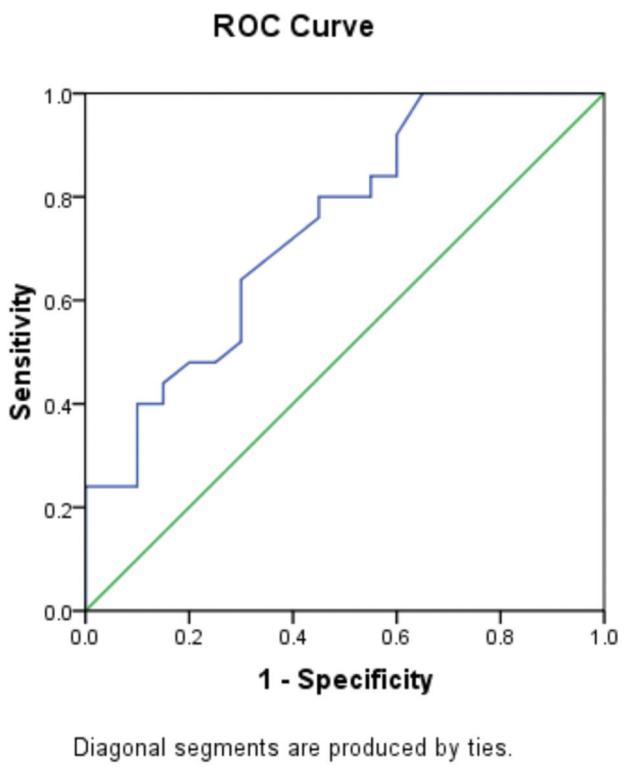
Despite the importance of the synovial fluid as an area for the immunological process in RA and hence its value as a biomarker pool, the clinical field is in constant need to detect vital indicators with the least possible invasive and reliable method. The fact that assessment in the peripheral blood is not as sensitive as assessment of inflamed target tissues does not negate the fact that the circulation clearly represents an important area for pathogenic studies, where studies in systemic diseases characterized by prominent of B-cell disturbances and autoantibody production have shown differences in certain peripheral B-cell subsets compared with healthy controls [19]. RF and anticitrullinated protein antibodies (ACPA) are produced by B cells; they activate complement and form immune complexes that become deposited in the joints, causing inflammation [20]. Nevertheless, they have considerable limitations; ACPA has extensive clinical applications as a more specific RA marker than RF and shows antibody positivity in cohorts of early synovitis in the range between 40 and 71%

Table 2 Demographic and laboratory results of the rheumatoid arthritis patients in terms of disease activity stages

Parameters	Remission group (*N*=5) (four women, one man)	Moderate group (*N*=13) (11 women, two men)	Severe group (*N*=7) (seven women)
Age (years)	42.2±6.30	43.92±11.58	45.14±11.89
Disease duration (years)	8.4±3.91	7.23±4.49	8.42±9.27
Disease activity score	2.59±0.49	4.5±0.59	6.45±0.28
BCDF (pg/ml)	130±29.81[a]	214±24.44[a]	306±40.57
MCSF (ng/l)	230±54.57	141±31.74	107±39.92
IL-2 (pg/ml)	53.8±19.45	46.1±7.21	72.5±14.08
IgG (mg/dl)	192±7.34[a,c]	224±3.51[a, b]	238±1.42[c]
ESR (mm/h)	23.50±5.37	47.23±8.69	37.0±10.44

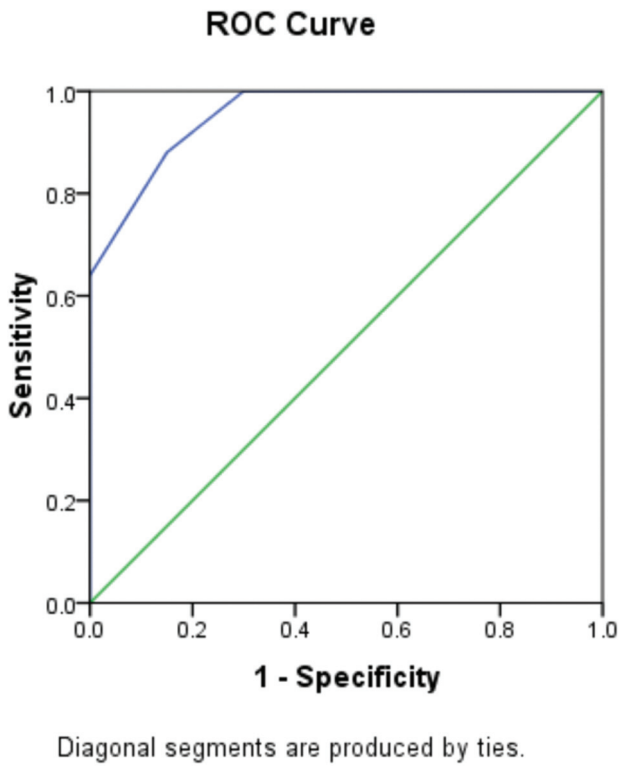
All data are expressed as mean±SE; BCDF, B-cell differentiation factor; ESR, erythrocyte sedimentation rate; IgG, immunoglobulin G; IL-2, interlukin-2; MCSF, macrophage colony-stimulating factor; [a]Significant difference from the severe group; [b]Significant difference from the remission group; [c]Significant difference from the moderate group.

Figure 1



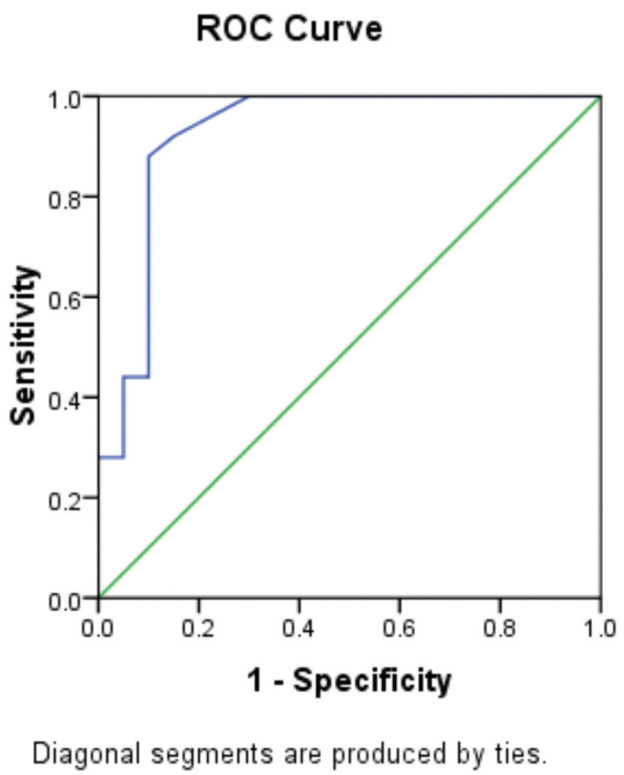
Receiver operating characteristic curve of B-cell differentiation factor among patients with rheumatoid arthritis. ROC, receiver operating characteristic.

Figure 2



Receiver operating characteristic curve of immunoglobulin G among patients with rheumatoid arthritis. ROC, receiver operating characteristic.

Figure 3



Receiver operating characteristic curve of macrophage colony-stimulating factor among patients with rheumatoid arthritis. ROC, receiver operating characteristic.

[21,22]. This may partly be because ~30% of RA patients never develops ACPA [23]. Moreover, there are subgroups of patients who are susceptible to joint damage, but test negative for both ACPA and RF; thus, the identification of novel autoantibodies is important in patients who lack RF and ACPA [24]. Consequently, it has been suggested that multiple biomarkers would be more accurate than a single biomarker to assess the prognosis of RA by enabling detailed stratification of the disease [25].

The present study aimed to investigate a panel of biomarkers from the diagnostic aspect such as BCDF, MCSF, IgG, and IL-2, where our results showed a significantly higher level of serum BCDF, MCSF, and IgG among RA patients compared with the healthy controls. Thus, we consider BCDF and MCSF as potential serum biomarkers of RA, with the possibility of incorporating IgG, which cannot be relied on solely owing to the fact that it is a general inflammatory marker than RA specific. Our findings indicated a BCDF cut-off value of 102 pg/ml with an accuracy of 0.925, an MCSF cut-off value of 37.5 ng/l with an accuracy of 0.741, and an IgG cut-off value of 195 with an accuracy of 0.955 for discrimination between healthy participants and RA patients. Our

findings are in agreement with those of Tobón *et al.* [19], who observed that RA is characterized by prominent autoantibody production in comparison with healthy controls, whereas increased levels of B-cell activating factor have been detected systemically in RA patients [26,27]. Samuels *et al.* [28] found a 3.4-fold increase in new emigrant/transitional B cells in the peripheral blood in patients with RA compared with controls. They attributed their findings to defective central and peripheral B-cell tolerance checkpoints in RA, resulting in the accumulation of a large number of autoreactive B cells in the mature naive B-cell compartment [28]. In addition to B-cell changes in RA, the role of GM-CSF in RA has also been studied, owing to its important role in mediating the function of T-cell in inflammatory conditions [29,30]. Davis *et al.* [31] reported that GM-CSF and its receptor levels were high in plasma and synovial fluid of RA patients, and GM-CSF receptors are overexpressed in the circulating mononuclear cells in the synovial tissue. M-CSF is among four other markers (tumor necrosis factor receptor superfamily member 9, transforming growth factor-α, CCL23, and CXCL13) showing a correlation with the level of C-reactive protein, RF level, and ESR; also, their plasma levels were shown to be good predictors of disease activity [32].

It is noteworthy that bone loss can occur before at very early stages of disease, whereas autoimmune processes [33,34] and molecular events [35] begin years before the clinical onset of disease, promoting joint destruction and disability, rather than just synovitis that needs enough time to cause the destruction of bone. Several studies have studied B cells' behavior in the early stages of RA, where B-cell intervention and disturbances in circulating memory B cells have been documented in patients with less than 6 weeks' disease duration [36] in addition to increased levels of cytokines as a result of B-cell activation and survival [37,38]. In the ESPOIR cohort, a large French multicenter prospective study, serum markers of B-cell activation, such as levels of IgA, IgG, β2-microglobulin, and free light chains of Ig, were found to be increased in early RA less than 6 months' duration compared with undifferentiated arthritis [39]. Although the current study is a cross-sectional one that included patients at different stages of the disease and different disease durations, yet, study of the events in the early stage of the disease was not our objective. In addition, none of our studied patients was known to have RA for less than 1 year; consequently, the current study did not present a perspective for BCDF and GM-CSF at early stages of the disease. Nevertheless, on grouping our patients according to disease activity into moderate and severe groups, we did not find an association between disease duration and activity, this finding boosts the concept that the disease activity does not necessarily time-dependant. Owing to the heterogenic nature of RA, as not all patients show progression to joint damage, there is an imperative unmet need for the identification of patients susceptible to joint destruction for early therapeutic intervention [25]. Accordingly, a protein biomarker signature comprising elevated levels of autoantibodies could stratify early-stage RA into distinct molecular subsets predictive of disease severity [40], which would support informed clinical decisions for individualizing treatment regimens [24].

On testing the fitness of our studied biomarkers in disease staging according to severity, we found that BCDF could differentiate remission and moderate stages from the severe stage of the disease, whereas IgG differentiates the three groups (remission, moderate, and severe) from each other; yet, we cannot recommend only IgG as it is not a specific associate to RA. This finding is should be investigated further to test the possibility of incorporating BCDF into the DAS as the latter lacks a biologic biomarker. Of equal importance to the proinflammatory factor in autoimmune diseases is the regulatory factor as the functional cross-talk between T regulatory lymphocytes (Tregs) and T effector lymphocytes (Teffs) is essential for the maintenance of immune homeostasis and is carried out by homeostatic cytokines, among which is IL-2 [41]. From earlier studies revealing decreased IL-2 production, synovial fluid, peripheral blood of RA patients [42] passing through general agreeability about the anti-inflammatory activity of IL-2 in autoimmunity, on the basis of the findings of inherent defects in the IL-2 pathway, decreased response to IL-2, and/or reduced IL-2 availability, resulting in the impairment of Treg function, survival/or frequency, in many autoimmune disorders in mice [43,44] and humans [45,46], recent studies have shown that decreased IL-2 serum levels could serve as biomarkers of disease progression [41,47]. In contrast with the previous results, we could not find a significant decrease in IL-2 in RA patients compared with healthy controls, nor a correlation with disease severity, which warrants further investigation on a larger group of RA patients, with a special focus on early disease stage.

Conclusion

Thus, we concluded from this study that BCDF, and MCSF and IgG could serve as reliable diagnostic biomarkers for RA and similarly, BCDF and IgG

for disease activity; therefore, we recommend proof-of-concept studies for the incorporation of BCDF and IgG into the disease staging systems as in the DAS.

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Nil.

Conflicts of interest
There are no conflicts of interest.

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