Reviewer's report

Title: Lymphocyte and monocyte flow cytometry immunophenotyping as a diagnostic tool in uncharacteristic inflammatory disorders

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Reviewer: Jan Ehrchen

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Major Compulsory Revisions

General: The authors present a descriptive study accessing the use of immunophenotyping of lymphocytes and monocytes as a diagnostic tool in uncharacteristic inflammatory disorders. They especially focused on cell surface expression of CD40 on monocytes and cell surface expression of MHC-II on CD4 and CD8 positive T cells. Patients suffering from acute gram-negative septicemia, EBV or influenza and patients suffering from tuberculosis, neuroborreliosis or collagenosis were included in the study. The data are sound and the comparative analysis of monocyte and T-cell activation in diverse infections and autoimmune disorders is of interest. However, the author's conclusion that bacterial and viral infection, as well as systemic autoimmunity can be discriminated by the described immunophenotyping approach is not justified by the provided data in its present form.

1.) The question whether differences in phenotypic marker expression between the different inflammatory conditions were statistically significant was not addressed. As example, regarding the diagnostic value of CD40 expression on monocytes and MHC-II expression on T cells the authors state on the first page of the discussion that “an intermediate CD40 elevation in monocytes plus a large fraction of activated cells among the CD4+ T helper cells was seen only in the subacute bacterial tuberculosis and borrelia infections” and “a similar pattern was seen among the collagenosis patients, however, with same levels of activation in CD4+ T cells as in CD8+ T cells” This conclusion is not based on statistical analysis. With respect to the results depicted in Figure 1 and Figure 4 there is no significant increase in MHC-II expression on CD4 T cells from patients suffering from borrelia infection and also no significant increase in MHC-II expression on CD8 T cells from collagenosis patients. No statistical comparison between the collagenosis, borrelia infection and tuberculosis group was performed.

In general, in the present form of the manuscript only differences between inflammatory conditions and controls were evaluated. Moreover and quite important the number of patients analyzed in each subgroup was not provided (with the exception that 15 patients suffering from tuberculosis and borreliosis were analyzed according to page 3 of the discussion). This information however is vital to access whether the depicted differences are relevant. Moreover, in addition to the numbers of patients analyzed more information on the patient collective like sex and age distribution, medication etc. is needed if this method
should really be established as diagnostic tool. With respect to the number of tuberculosis and borreliosis patients the numbers are far too low for evaluation of a procedure for clinical diagnostic. If this is the aim of the present study the help of a statistician could be very helpful.

2.) The authors aim at developing a diagnostic tool for patients with uncharacteristic inflammatory symptoms like prolonged fever or longstanding fatigue. However, the most prominent differences in activation marker expression were found in patients suffering from rather acute infections (less than 2 weeks as stated in the Methods section) with probably characteristic symptoms like EBV infection and gram negative septicemia. Thus, the differences in activation marker expression could be due to acute versus chronic inflammatory conditions. Thus, an inclusion of a chronic viral infection like CMV or both the acute and chronic phase of autoimmune disorders would be helpful for a better understanding of the observed differences. At least this point has to be critically discussed.

3.) The authors should comment on their choice of T cell and monocyte activation markers. With respect to T cell activation markers the authors state that they did not observe high enough mean fluorescent intensity for CD25 expression (used in combination with foxp3 as marker for regulatory T cells). Did they check for increased expression of CD69 and CD44 or for low expression of CD62L? With respect to monocytes did the authors analyze other costimulatory molecules like CD80 and CD86 or markers of alternative activation? Did the CD14+CD16+ monocytes also express CD40 and did the authors find differences in their CD40 expression in the inflammatory conditions analyzed?

4.) The authors conclude that their immunophenotype patterns provide superior information compared to more simple-to-assay parameters like CRP. As example they state that there was a CRP of more that 100 mg/dl in some influenza patients and of lower than 100 in 15 subacute bacterial tuberculosis and borreliosis patients, thus CRP was not able to discriminate between bacterial and viral infection. Why does this indicate that CRP values are not suited to discriminate between these conditions? Moreover, to reliably assess whether the immunophenotype patterns introduced by the authors are superior to routine parameters (or combinations of routine parameters) statistical methods have to be applied (including multi parameter comparisons).

5.) Independent from the question whether the observed immunophenotype patterns could really be used as a new diagnostic tool this study represent the first comparative analysis of MHC-II expression on CD4 and CD8 T cells in combination with the analysis of both monocyte cell surface and soluble activation markers and monocyte subsets in diverse infections and autoimmunity. The authors could focus on this finding and discussed it in more detail. For instance the authors should include the literature regarding activation induced up-regulation of MHC-II on T cells and discuss the potential functional relevance of this phenomenon and of monocyte activation in the conditions analyzed.
1.) Discussion page 13, 2. paragraph, line 3: include mg/dl after the CRP-values
2.) results, page 9, 3. paragraph line 2: correct CD14- to CD14+

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**

I declare that I have no competing interests.