Grp94 [9], whereas SDS at the concentrations used by authors was ineffective. The abnormally elevated dispersion of data even in healthy subjects, who instead should not show any positivity for autoantibodies, might thus originate from non-specific binding of non-immune IgG in any serum sample to antigen Grp94. The same troublesome aspect likely also occurs with Bip, since Bip is even more involved than Grp94 in binding IgG [10].

Another aspect that might contribute to the larger-than-expected variability of data (Table 1, Fig. 3 in the paper) is the lack of stratification of patients, especially for those analysed twice. It was of crucial importance to group patients for variables (such as the presence of other pathologies, type and duration of therapies, and plasma concentration of IgG), that can affect clinical evolution of disease, causing either a decrease or increase in the level of anti-Grp94 antibodies. Lack of information about relevant clinical and bio-humoral parameters on patients at the two separate visits makes the statistical analysis (Tables 2 and 3 in the paper) a purely mathematical exercise without any biological meaning. Changes in the mean values of both anti-Grp94 and anti-Bip antibodies in RA patients at Visit 2 compared with Visit 1 are evident in both Figs 3 and 4 in the paper (redundant), suggesting that some variables actually intervened in time in modifying the values of antibodies in the same patients. Strangely, however, the authors omitted to stress this difference, stating that the level of antibodies remained constant.

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Comment on: Antibodies to the endoplasmic reticulum-resident chaperones calnexin, Bip and Grp94 in patients with rheumatoid arthritis and systemic lupus erythematosus: reply

Sir, We appreciate the interest of Tramentozzi et al. in our work [1] and would like to respond to their comments. Tramentozzi et al. [2] questioned our ELISA system for detection of antibodies to endoplasmic reticulum (ER)-resident chaperones in serum of patients with RA and SLE. In the following, we explain that our ELISA system is highly reproducible and well established: first, negative and true-positive samples were clearly identified and verified by immunoblot. Secondly, inter-plate correlation was assessed both by carrying along one defined negative as well as one positive serum as reference on each plate. The ratio of these values allowed normalization of each serum for individual anti-ER chaperone antibody titres. Thirdly, sera were analysed in duplicate. Fourthly, an a priori test of several dilutions of sera was performed to determine the linear range and the optimal dilution factor (1:40) providing optical density (OD) values within the linear range. The dilution factor was kept constant to enable statistical evaluation. In this kind of investigation, values expressed as fold induction in comparison with healthy individuals are as meaningful as values expressed in antibody titres. However, for obvious reasons it is not feasible to exactly quantify the concentration of antigen-specific antibodies within polyclonal sera containing multiple antibody clones of various isotypes and immunoglobulin G (IgG) subclasses binding with different affinities and avidities,

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prior to onset of a chronic autoimmune disease [15].

To our knowledge, there is no specific hierarchy prescribing a distinct chronological order of ELISA and western blot analyses. Unlike Tramentozzi et al. [2], we think that it is indeed critical to verify the specificity of antisera tested in ELISA by immunoblotting using the same antigen preparation, because bands were detected at the expected molecular weight and corresponded to results using defined monoclonal antibodies as a reference, thus confirming a specific reaction.

Tramentozzi et al. [2] further claim lack of stratification of our patients. Patients have been clinically very well characterized and grouping of patients for different variables was carefully done. Nevertheless, no correlation between any clinical parameter with the generation of anti-ER chaperone antibodies could be determined. Changes in the mean value of anti-ER chaperone antibodies are only visible in Fig. 4 [1]; where antibody titres are shown for each patient at different visits by solid lines, this information is not included in Fig. 3 [1].

In summary, the methodology used in our study was suitable to investigate the addressed questions and thorough evaluation of data allowed us to conclude that elevated autoantibody titres against Grp94, BIP and calnexin arise from specific autoimmune reactions in RA and SLE patients and are not a stochastic problem.

Disclosure statement: The authors have declared no conflicts of interest.

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References


Roth et al.

SIR, We read with interest the recent article by Rubbert-III randomized study (MIRROR) with active rheumatoid arthritis: results of a Phase treatment dosing regimens of rituximab in patients

Comment on: Efficacy and safety of various repeat treatment dosing regimens of rituximab in patients with active rheumatoid arthritis: results of a Phase III randomized study (MIRROR)

Sin, We read with interest the recent article by Rubbert-Roth et al. [1]. The MIRROR trial provided an opportunity to answer an important clinical question: is there a difference in efficacy between lower doses and higher doses of rituximab in the treatment of RA?

Bias is widespread in the medical literature and we note several issues in the published report of this study that are of concern and potentially misleading.

(i) Although the design of this study is stated to be a randomized, double-blinded controlled trial, the process was reported as flawed by the authors, resulting in a biased study and incorrect treatment allocation to some of the participants [1]. A more detailed and clear explanation of exactly what happened and how this affected the treatment allocation, binding process and results is needed.

(ii) The whole purpose of the complex process of blinding and randomization involved in such a study design is to reduce or eliminate both covert and overt bias in so far as is possible. Therefore, the analyses that adjusted for baseline factors as reported raises serious concerns about the design, process, analysis and results, as the analyses should be straightforward with such a design.

(iii) Figure 2 reports that some patients received placebo. Nowhere in the ‘Methods’ section is there a statement about a placebo being administered in this trial.

(iv) Although the authors used an as-treated analysis instead of an intention-to-treat analysis, we believe this is not acceptable and those who were incorrectly assigned should have been excluded from the analysis. An intention-to-treat analysis is favoured as treated is the statement about a placebo being administered in this trial.

We believe in the interest of fair and honest reporting that this article should be amended. Additional details should be provided on exactly what happened during the treatment allocation process, whether a placebo was

References


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Comment on: Efficacy and safety of various repeat treatment dosing regimens of rituximab in patients with active rheumatoid arthritis: results of a Phase III randomized study (MIRROR)

In summary, we have several concerns about this report. We believe in the interest of fair and honest reporting that this article should be amended. Additional details should be provided on exactly what happened during the treatment allocation process, whether a placebo was