10 YEARS OF BREAKTHROUGHS A HEALTHIER FUTURE

PRECISESADS
Molecular Reclassification to Find Clinically Useful Biomarkers for Systemic Autoimmune Diseases

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New bio-informatic analysis of flow cytometry data.

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Facts & Figures

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Challenge

Systemic autoimmune diseases (SADs), such as Lupus or Rheumatoid Arthritis, share clinical manifestations and pathophysiological mechanisms making diagnosis difficult. The PRECISESADS project launched with the aim to reclassify SADs by finding useful biomarkers. To achieve this goal, multiple laboratories across Europe included around 3000 patients and controls to generate OMICs data (genomic, proteomic, epigenetic,..).

Among the OMICs data, our work focused on the generation of calibrated and normalized data related to the flow cytometry analyses of peripheral blood cells targeted with fluorescent dye-associated antibodies. To achieve an in-depth identification and numeration of the immune cell populations and the fine characterization of their cell surface expressed proteins using their Mean Fluorescence Intensity (MFI), difficulties arise when comparing the results obtained in the different centers using distinct flow cytometers.

Approach & Methodology

The first step was to warrant the stability of the instruments during all the project. To fulfill this, we used standardized 8 peak beads before all flow cytometry acquisitions. Those beads produce 8 peaks of increasing fluorescence intensity. Matching the MFI of each peak to a reference yield to verify the cytometer stability. A normalization script has been elaborated using linear regression on the 8 peak MFIs to adjust the fluorescence intensities if needed. This normalization has been assigned to the cell data.

Furthermore, MFI variations were observed due to batch effects from the different lot of fluorescent antibodies used. A correction has been applied thanks to a coefficient that equalize the median of the different batches. This correction has been assigned to the cell data.

The second step was to make the MFIs comparable between all cytometers. A center effect was corrected using a coefficient for each fluorochrome from each center. This coefficient was computed with a ratio between the median of all cell marker MFIs obtained with one instrument and the medians from a reference instrument.

Results

The normalization procedure reduces the technical variability resulting from a shift between the 8 peak beads and their reference. This was done by modifying each channel independently using the 8 peak bead reference values (Figure 1).

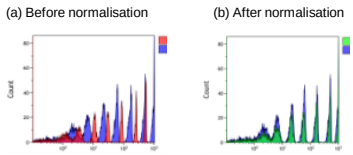

Figure 1. Representative example of the normalisation script. (a) The 8 peak beads from one fluorsence channel (red) are compared to the reference original data (blue). (b) normalized data (green) are compared to the reference peaks (blue).

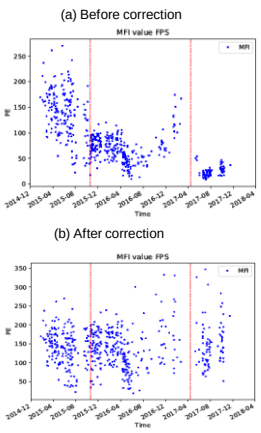

Figure 2. Representative example of the PE MFIs obtained according to the date of acquisition from one cytometer. The batch effect observed before correction (a) is abrogated after correction with a specific coefficient (b).

According to the acquisition date, a batch effect can be observed associated to the lot of the fluorescent antibodies used (Figure 2). The red lines indicate the date of data acquisition with new lot of antibodies. We extracted the median values of the MFIs from the three batches. A coefficient was computed to obtain median values corresponding to that of the first batch. This calculation has been done for each dye in each cytometer. Each coefficient was then apply to the corresponding files to abrogate the antibody batch-dependent effects.

A center effect was observed when comparing the MFI values (Figure 3). This effect was not observed with the population frequencies calculated by all cytometers. This suggested that a specific correction was required for each dye and for each cytometer to allow the inter-cytometer comparison (Figure 4).

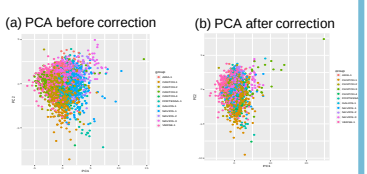

Figure 3. PCA done on the MFI values all fluorescence dyes before (a) and after (b) application of the correction between centers.

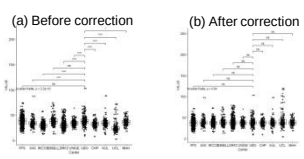

Figure 4. Representative example of one fluorescence dye in all centers before (a) and after (b) application of the correction.

Value of IMI collaboration

The coordination of many laboratories allowing the inclusion of thousands of patients in Europe and the generation of a large amount of flow cytometry data would have not been possible without the collaboration of IMI.

Impact & take home message

This complex normalisation procedure ensures that the flow cytometry data can be safely used with the other OMICs data from PRECISESADS project to succeed in the reclassification of the SADs.

imi innovative medicines initiative efpia

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