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Blood proteins linked to immune tolerance, inflammation and cellular junctions reveal a succession of pathophysiological events preceding the relapse in Crohn's disease patients stopping infliximab

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Introduction

In Crohn's disease (CD), biologics well succeeded in inducing mucosal healing and stable remission. When this objective is achieved, treatment de-escalation is considered and the relapse constitutes the main risk of such choice. In the context of biologics withdrawal in CD, risk factors predicting the relapse have been intensively searched. Although these investigations classically respond to a practical need, we recently highlighted that they can also serve to better understand the underlying mechanisms of relapse. Indeed, the comprehension and the prediction of relapse are two sides of the same coin. By stratifying CD patients according to time to relapse, we identified distinct pathophysiological processes associated with the risk of short-term (< 6 months) and mid/long-term (> 6 months) relapse after stopping anti-TNFα treatment. In our previous study, we did not target proteins specifically related to CD since we used the biomarkers discovery workflow which is a hypothesis-free method. This approach combined with the low analytical sensitivity of our measurement method (selected reaction monitoring) led us to mainly measure the highly abundant and liver-produced proteins of the serum. As a complement to this strategy, targeting serum proteins less abundant and more specifically related to the development of CD could improve the understanding and the prediction of relapse after anti-TNFα withdrawal.

Aim

To test whether circulating proteins, implicated in the immune response, can provide novel insights in the comprehension and the prediction of relapse in CD patients stopping anti-TNFα.

Methods

We used a proximity extension assay (PEA) panel (Olink) targeting 92 proteins involved in the immune response. These proteins were measured in the baseline serum of patients belonging to the cohort of infliximab diScon-Tinuation in CrOhn's disease patients in stable Remission on combined therapy with Immunosuppressors (STORI, n=102). To investigate the dynamic of the markers before the relapse, we stratified the cohort according to a time to relapse of 6 months. All the subsequent analyses were performed in the short-term relapse (<6 months), mid/long-term relapse (>6 months) and non-stratified datasets. Association of markers with the risk of relapse was determined with the univariate Cox model. By using Uniprot, Human Protein Atlas and literature, we systematically characterised the function and the cellular origin of each measured protein. According to this database and the statistical analysis, we searched for biological patterns differentiating the short-term from the mid/long-term relapsers. To evaluate the capacity of our markers to jointly predict the relapse, we combined them systematically by pairs (with the 'AND' or 'OR' logical operators) and used the log-rank statistic.

Results

The risk of short-term relapse was associated with a decreased circulating level of proteins belonging to the inflammatory pathways while an increased circulating level of those markers was associated with the risk of mid/long-term relapse. The risk of short-term relapse was specifically associated with an increased circulating level of interleukin-6 (IL-6). The risk of mid/long-term relapse was specifically associated with a decreased circulating level of proteins showing anti-inflammatory properties: interleukin-10 (IL-10); corticosteroid 11-beta-dehydrogenase isozyme 1 (HSD11B1).
The risk of short-term relapse was specifically associated with a changed circulating level of proteins involved in tolerance and immunity of antigen presenting cells (APCs) (Allergin-1: MILR1; C-type lectin domain family 4 member C: CLEC4C; CLEC4G, CLEC4A; CLEC7A; lysosome-associated membrane glycoprotein 3: LAMP3).
The risk of mid/long-term relapse was specifically associated with an increased circulating level of proteins implicated in lymphocytes tolerance (lymphocyte activation gene 3 protein: LAG3; signaling threshold-regulating transmembrane adapter 1: SIT1; SH2B adapter protein 3/ SH2B3) and a decreased circulating level of cellular junction proteins (contactin-associated protein-like 2: CNTNAP2; corneodesmosin: CDSN; coxsackievirus and adenovirus receptor: CXADR; integrin α-11: ITGA11).
We found 1046 (short-term relapse dataset), 233 (mid/long-term relapse dataset) and 99 (non-stratified dataset) novel marker combinations which showed FDR<0.05 and higher Z-scores than C-reactive protein (CRP) and faecal calprotectin.

Conclusions

By studying blood proteins, we discovered that immune tolerance (in lymphocytes and APCs), inflammation and cellular junctions are dynamically modulated before the relapse of CD patients stopping anti-TNFα. These findings could help to better understand the underlying mechanisms of relapse. Compared to CRP and faecal calprotectin (the current references for relapse prediction), our novel marker combinations showed a high capacity to predict the relapse thus highlighting their potential for a clinical application.