

## LETTER TO THE EDITOR

Iran J Allergy Asthma Immunol

April 2020; 19(2):206-208.

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# Autologous Whole-blood Injections in Chronic Spontaneous Urticaria: Assessment of Efficacy Biomarkers

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Received: 2 July 2019; Received in revised form: 29 November 2019; Accepted: 3 December 2019

## To The Editor

Second-generation antihistamines are suggested as a first-line therapeutic approach in chronic spontaneous urticaria (CSU), with omalizumab or cyclosporine being proposed in case of refractoriness. For selected cases, due to ineffectiveness, dose-related adverse side-effects or lack of availability of the aforementioned treatment options, alternative therapeutic modalities, including autologous whole blood injections (AWBI), revealed an additive benefit in CSU treatment and are still used in clinical practice.<sup>1</sup> As regards disease's pathogenesis, an imbalance in cytokine expression has been demonstrated previously in patients' plasma and/or serum, including upregulation of Interleukin(IL)-12p70, IL-6 expression and down-regulation of IL-10 and interferon(IFN)- $\gamma$ , thus suggesting a mixed Th1/Th2-profile in CSU.<sup>2,4</sup> This was also accompanied by increased Th17-expression<sup>5</sup> or decreased levels of cytokines suppressing Th1/Th17 immune responses (such as IL-10 and IL-35).<sup>4,6</sup>

Of note, studies to determine cytokine expression correlated with skin homeostasis, such as IL-34, are scarce in CSU. Furthermore, although IL-27 has been implicated in the pathogenesis of other chronic inflammatory dermatoses, such as psoriasis, its role in CSU remains unknown.

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Previously, we have shown a favorable response in 19 adult CSU-patients following AWBI-intervention, by means of improvement in clinical symptoms and quality of life, as assessed by urticaria activity score/(UAS)7, dermatology-life-quality-index/(DLQI), chronic urticaria-quality of life questionnaire/(CU-Q<sub>20</sub>L) alterations at baseline, 8 and 12 weeks post-AWBI.<sup>7</sup> AWBI was administered intramuscularly for 8 weeks, on a weekly basis. Patients were grouped as responders in case of improvement of more than 14 points in the UAS7 and as non-responders otherwise.

Our study aimed to investigate biomarkers in the peripheral blood predicting the effectiveness of AWBI in CSU and to potentially unravel underlying pathogenetic mechanisms. We longitudinally studied changes in patients' plasma cytokine levels during AWBI-treatment compared to respective levels of a matched healthy-control-group at baseline. Determination of protein expression was performed using Luminex assays for IFN- $\gamma$ /IL-6/IL-10/IL-12p70/IL-13/IL-27/IL-34/IL-35 (Millipore Inc., Billerica, MA, USA). Study procedures were approved by the local tertiary University-hospital ethics-committee (Approval-Number:4418/03-10-2016). All participants fulfilled written informed consent.

Patients and controls did not differ with respect to age ( $p=0.2$ ) and gender ( $p=0.73$ ). IL-6/IL-13/IL-34/IL-35 measurements were below the detection limits of the assays and thus, not used for further analyses. Regarding IL-10/IL-12p70/IFN- $\gamma$ /IL-27 although

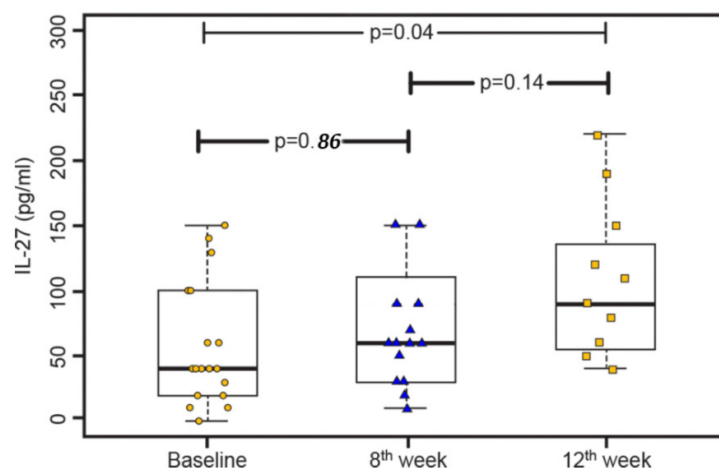


Figure 1. Patients' IL-27 plasma levels (pg/ml) at baseline, 8<sup>th</sup> and 12<sup>th</sup> week.

plasma-samples were collected in all predefined time-points, they were finally determined in 40/57 and 15/17, 38/57-11/17, 36/57-16/17, 43/57-17/17 samples of patients and controls respectively.

Baseline IL-12p70 levels did not differ between patients and controls ( $p=0.48$ ). Patients with favorable response to AWBI presented increased baseline IL-12p70 plasma-levels compared to non-responders ( $56.48 \pm 13.71$  vs  $34.79 \pm 12.5$  pg/mL respectively/ $p=0.01$ ). Significant associations were noted between baseline IL-12p70 levels - UAS7 scores (Spearman rho/ $r_s=0.660/p=0.01$ ) and UAS7 changes ( $r_s=0.737/p=0.004$ ) at the 8<sup>th</sup> week post treatment (responders: $\Delta$ UAS7= $-35/IQR=8.75$ , non-responders: $-14/IQR=7$ ).

Baseline IFN- $\gamma$  levels were lower in patients compared to controls ( $0.745/IQR=0.85$  vs  $1.16/IQR=1.43$  pg/mL, respectively/ $p=0.02$ ); however, these were not associated with the level of therapeutic response ( $p=0.32$ ).

AWBI-responders presented a significant increase in IFN- $\gamma$  expression from baseline to 8<sup>th</sup> week ( $\Delta$ IFN- $\gamma$ :  $0.62 \pm 0.46$  vs  $-0.56 \pm 0.88$  pg/ml, responders vs non-responders respectively/ $p=0.02$ ). Enhanced IFN- $\gamma$  expression post-intervention (8<sup>th</sup> week) was associated with significant improvement in UAS7 and CU-Q2oL ( $r_s=0.698/p=0.017$ -Pearson  $r=0.649/p=0.031$ , respectively). IFN- $\gamma$  expression gradually diminished following ABWI-discontinuation, even below baseline

levels at 12<sup>th</sup> week, both in responders (8<sup>th</sup> week: $0.95/IQR=0.86$ , 12<sup>th</sup> week: $0.35/IQR=0.61$  pg/ml) and non-responders (8<sup>th</sup> week: $0.84/IQR=0.76$ , 12<sup>th</sup> week: $0.25/IQR=0.51$  pg/mL).

Baseline IL-27 levels were lower in patients compared to controls ( $57.2 \pm 46.7$  vs  $431.1 \pm 269.2$  pg/mL, respectively/ $p<0.001$ ). No association between baseline IL-27 values and treatment response was observed ( $p=0.67$ ). An increase in IL-27 expression post-AWBI was noted (8<sup>th</sup> week:  $66.42 \pm 42.53$ , 12<sup>th</sup> week:  $104.54 \pm 60.88$  pg/mL-Wilks' lambda= $0.403/p=0.026$ -paired comparisons between baseline/8<sup>th</sup> week, 8<sup>th</sup> week/12<sup>th</sup> week, baseline/12<sup>th</sup> week,  $p=0.86$ ,  $p=0.14$ ,  $p=0.04$  respectively) (Figure 1), without, however, differences between responders and non-responders ( $p=0.73$ ).

Baseline IL-10 levels did not differ between patients and controls ( $p=0.08$ ) or responders and non-responders ( $p=0.89$ ).

Notably, in subjects with a favorable response to AWBI, increased IFN- $\gamma$  expression at 8<sup>th</sup> week was strongly correlated with the following increase in IL-27 expression at 12<sup>th</sup> week ( $r_s=0.900/p=0.03$ ). In non-responders, a negative correlation between baseline IL-10 and IL-27 levels at 8<sup>th</sup> week ( $r_s=-0.975/p=0.005$ ) were noted.

It is well-known that IL-12 represents a key-mediator in the inflammatory response, resulting in IFN- $\gamma$  induction by T and natural killer (NK) cells.

Previous studies have suggested that increased pro-inflammatory cytokine milieu, including IL-12p70, is implicated in CSU pathogenesis.<sup>2</sup> In our study, we did not conclude any difference in respect to IL-12p70 expression between patients and controls.

Low IFN- $\gamma$  expression in our cases, is in accordance with previous studies,<sup>4</sup> probing an altered Th1-response in CSU; however, response to AWBI was associated with an increase in IFN- $\gamma$  expression post-intervention in responders.

It has been suggested that innate immune response is dysregulated in CSU, as indicated by the impaired response from plasmacytoid dendritic cells (pDCs).<sup>8</sup> Baseline levels of IL-27 -a pro-/anti-inflammatory cytokine, mainly induced by pDCs- were lower in patients compared to controls in our cohort, potentially reflecting the aforementioned pDCs dysregulation. The observed increase in IL-27 expression post-intervention along with the IFN- $\gamma$  increase in responders suggest possible crosstalk between DCs and IFN- $\gamma$ -producing cells (CD4/CD8T/NK-cells).

Experimental models investigating the role of NK cells-DCs interaction in the control of the adaptive Th17-response have demonstrated an amplifying feedback-loop between IL-27 and IFN- $\gamma$  production, attributing to the restoration of the dysregulated IFN- $\gamma$ -IL-27 axis.<sup>9</sup> The interdependence of IFN- $\gamma$  and IL-27 in a self-amplification model might also be relevant in the case of AWBI-responders, who presented an initial increase in IFN- $\gamma$  expression (8<sup>th</sup> week), followed by a subsequent increase in IL-27 levels (12<sup>th</sup> week).

Although previous reports have suggested that the IFN- $\gamma$ -IL-27 axis dysregulation could be attributed to increased IL-10 expression,<sup>9</sup> we did not verify significant alteration in IL-10 levels following the intervention.

Our study is mainly limited by the small patient sample and the lack of control group of patients treated with placebo. Nevertheless, our main strength is the longitudinal assessment of inflammatory profiles in CSU patients, by multiplex assays in relatively short intervals, thus reflecting the therapeutic effect more accurately.

AWBI-treatment was followed by restoration of the Th1 response in CSU patients, as indicated by increased IFN- $\gamma$  expression and a subsequent upregulation in IL-27 expression, possibly due to the induction/restoration of the dysregulated IFN- $\gamma$ -IL-27 axis. In this context, baseline IL-12p70 plasma levels

need to be further evaluated as a potential biomarker predicting effectiveness post-AWBI in CSU patients.

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