From Macro to Nano: Linking Quantitative CEUS Perfusion Parameters to CD4+ T Cells Subtypes in Spondyloarthtitis

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ABSTRACT

The onset and progression of immune-mediated inflammatory arthritis, such as rheumatoid arthritis and spondyloarthritis, are linked to the IL23-IL17 immune axis, so that many therapeutic strategies aim at modulating this pathway. However, there is so far no possibility of an *in vivo* direct monitoring, without a biopsy, of the specific T cells involved in this modulation. Synovial perfusion, and thus synovial angiogenesis, has been recognized as a sensitive and early marker of inflammation that can be evaluated via quantitative analysis of contrast-enhanced ultrasound imaging data.

We propose a quantitative analysis of contrast enhanced ultrasound data, exploiting both a pixel-wise analysis for characterizing the perfusion patterns and their heterogeneity within a patient's synovia, and a model that add to the gamma-variate function a term accounting for a possible slow-flow component, whose presence and amplitude is estimated via a variational Bayesian method.

We show that this quantification allows to find a relationship between perfusion parameters to CD4+ T helper cells subtypes that are believed to be involved in the IL23-IL17 immune axis modulation: significant correlations are as high as 0.90, suggesting the possibility of estimating T cells concentration from non-invasive imaging data.

Index Terms— Quantification, perfusion, contrast enhanced ultrasound, arthritis, synovitis, T helper cells, variational Bayes, single compartment recirculation

1. INTRODUCTION

The IL23-IL17 immune axis is thought to play a critical role, albeit not yet completely understood, in immune-mediated inflammatory arthritis, such as rheumatoid arthritis and spondyloarthritis. As a result, in the last 10 years drug research has focused on developing several therapeutic applications to modulate this pathway, aiming at preventing progression of chronic disease or providing its stable remission [1-3]. However, there is so far no possibility of an *in vivo* direct monitoring of the cytokines involved in this immune pathway, without a biopsy. A valuable alternative, not yet investigated, might be linking the cytokines expression levels with the noninvasive markers of inflammation, commonly used in clinics, which correlate with arthritis activity.

In particular, synovial perfusion, and thus synovial angiogenesis, has been recognized as a sensitive and early marker of inflammation that can be evaluated via quantitative analysis of contrast-enhanced ultrasound imaging data [4-5].

Contrast Enhanced Ultrasound (CEUS) is an imaging technique for the visualization and assessment of tissue vascularization and perfusion, using microbubbles as contrast agent. CEUS has been widely studied for the early detection and grading of arthritis diseases, since it allows a non-invasive assessment of the synovial neo-vascularization and of the variations in local perfusion [7-10]. In a previous work, we have tackled the CEUS quantification problem at the pixel level and we have demonstrated that pixel-wise quantification allows such an effective characterization of different perfusion patterns that they can be then used to discriminate between arthritis subtypes [11].

Here, we study the possibility of using a quantitative analysis of synovial perfusion parameters as *in vivo* markers of the modulation of immune-mediated inflammation. Differently from previous findings [6-7], the identified relationships such as to promise that these parameters can be used to estimate non-invasively the expression of specific T helper (Th17) phenotypes involved in the IL23-IL17 immune pathway.

Although the mechanism linking cytokine expression to synovial angiogenesis (and thus synovial perfusion) are mostly unknown, the non-invasive quantification of perfusion patterns and their heterogeneity paves the way for the study of this missing link.

2. MATERIALS

Eight consecutive patients who fulfilled the Classification of Psoriatic Arthritis (CASPAR) criteria were enrolled in this study. The patients underwent a contrast-enhanced ultrasound examination of the most inflamed knee and synovial fluid samples were obtained from patients with active knee arthritis and synovial fluid effusion.

The study was approved by the local Ethic Committee of the University Hospital of Padova (Italy) (number 52723; October 11, 2010), and written informed consent was obtained from each participant in accordance with the principles outlined in the Declaration of Helsinki, after being informed about the intent and the methodology of the study.

Synovial fluid samples were collected from the swollen knees. After centrifugation, the synovial fluid mononuclear cells were isolated, plated and incubated and immunostained. Mononuclear cells were treated with commercially available anti-CD4 antibodies. $CD4^+$ T cells were gated with two different approaches: physical characteristic of cells and expression of CD4 in the area of lymphocytes. FACS analysis was assessed as previously described to identify different $CD4^+$ T cells subtypes, that are linked to IL23-IL17 immune axis: $CD4^+$, $CD4^+IL23^+$, $CD4^+IL17A\text{-}F^+$, $CD4^+IL17A\text{-}F^+IL23^+$.

3. METHODS

Ultrasound imaging

Each subject underwent a 2-min CEUS study with a 7-MHz transducer US device (MyLab25, EsaOte, pixel size 0.6 mm, dynamic range between 45 and 63 dB, gain between 0 and 20 dB) equipped with Contrast tuned Imaging (CnTI; Esaote), after a bolus injection of 4.8 ml of sulfur hexafluoride-filled microbubbles contrast agent (SonoVue, Bracco International B.V., Netherlands). Gray-scale US (anatomical B-mode image) was acquired before the bolus to define the boundaries of the synovial tissue).

CEUS images were motion corrected and coregistered to the anatomical image as described in [11]. We also linearized the log-compressed data as in [12] and normalized each pixel curve by the maximum value in the image data, in order to have the data in the range [0; 1].

From the synovial boundaries, we derived an image mask that was then modified to include only pixels that showed a significant enhancement, considered as the difference between baseline value and peak value larger than a fixed threshold, set to 0.2 (20% of the data maximum value) based on previous studies.

Perfusion model

CEUS data are generally quantified at the region of interest (ROI) level, i.e. analyzing the time intensity curve (TIC) obtained by averaging all pixel TICs within a specific user-defined region. Pixel-wise quantification coupled with a physiologically motivated perfusion curve, such as the Gamma-variate model, can accurately quantify synovial perfusion and it is more flexible and rich than a mono-exponential or logarithmic model (generally employed for CEUS kinetic description [13]) to describe many heterogeneous patterns [11].

However, the heterogeneity of the CEUS kinetics can be such that even a Gamma-variate model does not properly describe the time courses. One possible source of this heterogeneity, generally ignored in CEUS data modeling, can be an apparent trapping of the microbubbles in newly formed vasculature, when characterized by high tortuosity [14]. This apparent trapping can also be explained by the limited temporal duration of the CEUS scan, that prevents the identification of a very slow washout from the data. We thus propose the use of a nested model for describing the perfusion, the single compartment recirculation model [15,16], where the original Gamma-variate was augmented with its integral to explicitly model the recirculation of the contrast agent. Under the assumption of additive noise, the vector of CEUS measurements $\boldsymbol{c_{pixel}}(t)$ is described by:

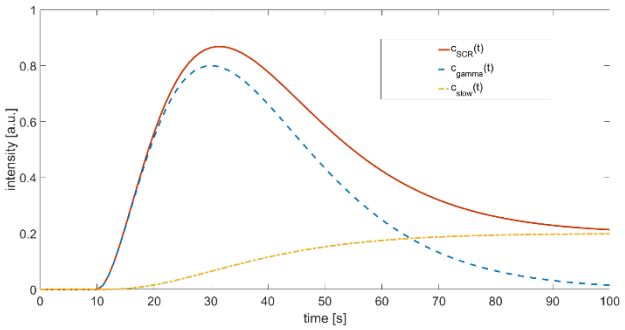

Fig. 1 Representative perfusion model (red solid curve) as combination of a gamma-variate component $c_{gamma}(p,t)$ (dashed blue curve) and an apparent trapping component $c_{SCR}(p,t)$ (dash-dot orange curve).

$$\boldsymbol{c_{pixel}}(t) = G(\boldsymbol{p},t) + \boldsymbol{e}(t)$$

where $G(\boldsymbol{p},t)$ is the model with the parameter vector $\boldsymbol{p}$ (which represents all the individual rate constants of the model) and $\boldsymbol{e}(t)$ is the measurement error. The nested SCR model is:

$$c_{gamma}(\boldsymbol{p},t) = \frac{a_0}{c_{gamma_{max}}} \cdot (t-t_0)^{\alpha} \cdot e^{-\frac{t-t_0}{\beta}} \quad t \geq t_0$$

$$c_{SCR}(\boldsymbol{p},t) = \frac{a_1}{AUC_g} \int_{t_0}^{t} c_{gamma}(\tau)d\tau \quad t \geq t_0$$

$$G(\boldsymbol{p},t) = c_{gamma}(\boldsymbol{p},t) + c_{SCR}(\boldsymbol{p},t)$$

being $\boldsymbol{p} = [a_0, \alpha, t_0, \beta, a_1]$ the parameter vector, where a_0 is a scaling factor, α and β determine the bolus shape (raise and washout of the dye from the vascular bed), t_0 is the contrast arrival time and a_1 is the scaling factor of the trapping component. From the set of estimated parameters $\hat{\boldsymbol{p}} = [\hat{a}_0, \hat{\alpha}, \hat{t}_0, \hat{\beta}, \hat{a}_1]$] it is possible to derive a set of additional macroparameters, such as the peak value G_{max}, the time of peak t_{max}, the raise time t_{raise} and the washout time t_{wash} (computed as the time needed to raise the intensity from the baseline value to half maximum and from the peak value to half maximum respectively), the mean transit time MTT, the

blood flow index BFI and the blood volume index BVI, for a total of 11 parameters for each curve.

Parameter estimation

The model is identified separately for each pixel using a Variational Bayesian (VB) estimator, as proposed in [17], which minimizes the difference between the posterior distribution of the parameters given the data and the model and its (computationally tractable) approximation.

We also incorporated the Automatic Relevance Determination (ARD) [18,19] within the algorithm to allow for the automated reduction of model complexity (from SCR to Gamma-variate model). This was achieved by using a shrinkage prior for the parameter to be subjected to ARD, that in our case was the amplitude a_1 of the irreversible component. The prior distribution for a_1 was Gaussian with zero mean and unknown variance (set initially very large, i.e. 106) with a Gamma hyper-prior on the variance of a_1 to be estimated from the data. The ARD variance becomes very small if the data does not support the extra complexity introduced by this parameter, so that the a_1 is set to zero as the model collapses to the simple Gamma-variate: a representative map of the model complexity (a_1 equal or different from zero) is shown in Fig. 3.

Parameter summarization

In order to describe the heterogeneity of distinct synovial flows as well as the presence of small areas of characteristic flow patterns of physiopathology significance, the distribution of each parameter over all pixels is summarized by four statistical descriptors: mean, standard deviation, 25th percentile and 75th percentile. This allows us to explore the importance of both the global perfusion patterns in the synovia and their variability. In particular, the distribution percentiles return information on the areas of the synovia characterized by the lowest or highest values of the parameters of interest, that can be important in case of localized foci of inflammation (Fig. 4).

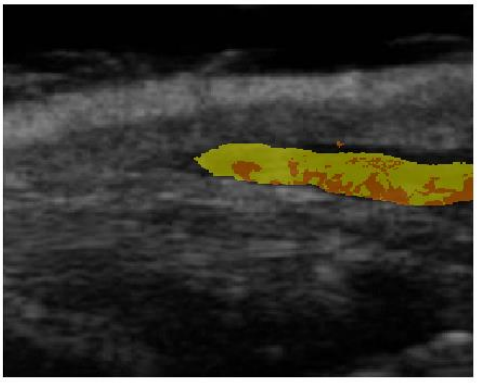

Fig. 2 Parametric map showing the model heterogeneity within a patient's synovium. In yellow the pixels where the automatic relevance determination (ARD) procedure estimated the presence of the Gamma component alone, and in orange the pixels where ARD estimate.

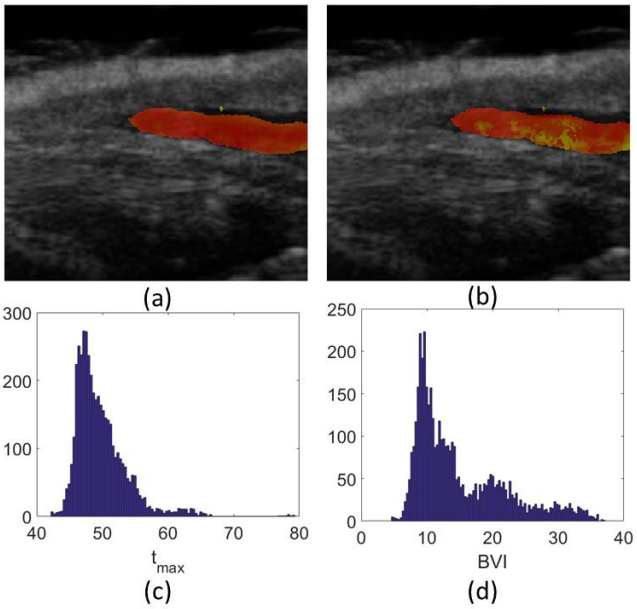

Fig. 3 Parametric map of the time-to-peak t_{max} (a) and of the blood volume index (b). From the maps and the correspondent distribution (c-d) it is clear that it is not always possible characterize an entire synovia by its mean value.

Statistical analysis

To verify the relationship between the non-invasive imaging perfusion patterns and the local levels in the synovia of Th17 cells, we evaluated the Spearman's correlation coefficient ρ between the parameters derived from the quantitative analysis of CEUS data and the expression levels of $CD4^+$, $CD4^+IL23^+$, $CD4^+IL17A\text{-}F^+$, $CD4^+IL17A\text{-}F^+IL23^+$. In order to accommodate for the scarcity of data, p-values of the correlations were computed using permutation test [20]. Correlations were considered statistically significant when the associated p-values were lower than 5% ($p<0.05$).

4. RESULTS

We found that the local expression levels of 3 different T cell lineages were significantly correlated with imaging perfusion estimates (ρ ranging from -0.9 and 0.81, p-value<0.05) (Table 1). In particular, the variability (as standard deviation) of the time of the maximum value reached through the scan, the contrast rate of appearance and the blood volume index were the most correlated with $CD4^+$ expression ($p<0.01$). The average value in the synovia of the mean transit time was interestingly highly correlated with the $CD4^+$ expression (ρ = 0.81, $p<0.05$) and its 25th percentile was inversely correlated with the $CD4^+$ $IL17^+$ $IL23^+$ levels (ρ = -0.85, $p<0.01$). The blood volume index was the only parameter correlated with all the cell lineages linked to the perfusion parameters. $CD4^+$ $IL23^+$ levels were not correlated with any CEUS estimates, probably due to the small values of the cell frequencies with respect to the $IL17^+$ subtype. In general, considering the variability of the perfusion imaging estimates led to higher correlations compared to the simple average descriptor,

highlighting the importance of a pixel-based approach where the full physiological description within the area of interest can be obtained.

		$CD4^+$	$CD4^+$ $IL17^+$	$CD4^+$ $IL23^+$	$CD4^+$ $IL17^+$ $IL23^+$
t_{max}	Mean				
	Std Dev	**-0.90***			
	25 prct				
	75 prct				
$peak$	Mean				
	Std Dev				
	25 prct				
	75 prct				**0.74**
t_{raise}	Mean				
	Std Dev	**-0.86***			
	25 prct				
	75 prct	**-0.75**			
MTT	Mean	**0.81**			
	Std Dev				
	25 prct				**-0.85***
	75 prct				
BVI	Mean		**0.71**		
	Std Dev	**-0.83***			
	25 prct				
	75 prct		**0.76**		**0.80**
a_1/a_0	Mean				
	Std Dev				
	25 prct				**-0.74**
	75 prct				

Tab. 1 Results on a selected set of perfusion parameters computed from the model fit. Only statistically significant correlations are reported ($p<0.05$, * for $p<0.01$).

5. CONCLUSIONS

We showed that the quantitative analysis of a non-invasive CEUS imaging scan can be used to determine the presence and the extent of local inflammation, that could be identified alternatively only with invasive biopsy (measuring Th17 expression levels).

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