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ORIGINAL ARTICLE

Vascular perfusion kinetics by contrast-enhanced ultrasound are related to synovial microvascularity in the joints of psoriatic arthritis

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Abstract The purpose of the study was to assess the relationship of the continuous mode contrast-enhanced harmonic ultrasound (CEUS) imaging with the histopathological and immunohistochemical (IHC) quantitative estimation of microvascular proliferation on synovial samples of patients affected by sustained psoriatic arthritis (PsA). A dedicated linear transducer was used in conjunction with a specific continuous mode contrast enhanced harmonic imaging technology with a second-generation sulfur hexafluoride-filled microbubbles C-agent. The examination was carried out within 1 week before arthroscopic biopsies in 32 active joints. Perfusional parameters were analyzed including regional blood flow (RBF); peak (PEAK) of the C-signal intensity, proportional to the regional blood volume (RBV); beta (β) perfusion frequency; slope (S), representing the inclination of the tangent in the origin; and the refilling time (RT), the reverse of beta. Arthroscopic synovial biopsies were targeted in the hypervascularity areas, as in the same knee recesses assessed by CEUS; the synovial cell infiltrate and vascularity (vessel density) was evaluated by IHC staining of CD45 (mononuclear cell) and CD31, CD105 (endothelial cell) markers, measured by computer-assisted morphometric analysis. In the CEUS area examined, the corresponding time-intensity curves demonstrated a slow rise time. Synovial histology showed slight increased layer lining thickness, perivascular lymphomonocyte cell infiltration, and microvascular remodeling, with marked vessel wall thickening with reduction of the vascular lumen. A significant correlation was found between RT and CD31+ as PEAK and CD105+ vessel density; RT was inversely correlated to RBF, PEAK, S, and β. The study demonstrated the association of the CEUS perfusion kinetics with the histopathological quantitative and morphologic estimation of synovial microvascular proliferation, suggesting that a CEUS imaging represents a reliable tool for the estimate of the synovial hypervascularity in PsA.

Keywords Contrast-enhanced harmonic ultrasound (CEUS) · Immunohistochemical (IHC) quantitative estimation ·

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Psoriatic arthritis (PsA) · Refilling time (RT) · Synovial hypervascularity · Vascular perfusion kinetics

Introduction

In psoriatic arthritis (PsA), vascular abnormalities represent the most significant histopathological changes in the synovial membrane (SM) and mainly in large joints, as first described by Espinoza et al. [1]. In knee joint synovitis of PsA as well as rheumatoid arthritis (RA), various types of changes in synovial microvascular architecture have been later confirmed by several other authors [2–4]. Typical macroscopic features of vascular architecture detected in the joints of PsA and other spondyloarthritis (SpA) consist in the high density of tortuous blood vessel network, appearing right from the early phases [5, 6], and persist throughout the relapsing course of these diseases [7–9].

The increased vascularity, showing as an increase in the number of discrete vessels, represents one of the basic macroscopic parameters used to define the intensity of synovitis, and microvessel density is commonly used as a surrogate measure for angiogenesis [10]. Synovial neovascularization, both in joint- and teno-synovial lining, has been also showed associated with increased expression of different adhesion molecules, cytokines, and chemokines [11–13]. In particular, high expression of vascular endothelial growth factor receptors, as angiopoeitin-2 ligand of receptor tyrosine kinase with immunoglobulin-like/EGF-like domains (Tie) 2, representing two major pathways of angiogenesis and blood vessel remodeling, has been demonstrated at level of capillary endothelial cells and synovial tissue of PsA and other SpA [14–16]. Further, interleukin (IL)-1ß and IL-6 in synovial fluid (SF), as the $CD45^+$ mononuclear cells (MNCs) and $CD31^+$ vessels in synovial tissue, have been also reported as candidate synovial biomarkers in PsA [17].

The progress in the field of not-invasive imaging techniques has changed the diagnostic approach to inflammatory arthropathies, and magnetic resonance imaging (MRI), dynamic contrast-enhanced (DCE) MRI [18, 19], and ultrasonography [20] are today considered key for the assessment of synovial proliferation and vascularity. MRI measures of synovial volume and rate of synovial enhancement have demonstrated a good correlation with macroscopic and microscopic synovitis scores in arthritis patients [18, 19]. DCE MRI is now considered to be the most valuable method for monitoring synovitis, also allowing the depiction of different shapes and distributions of time-intensity curves in arthritis [21]. Additionally, a fast enhancement, followed by early washout by DC MRI, has been identified as the predominant feature in knees of patients with early RA [22].

However, differences between gadolinium, which is known to diffuse to extravascular space and microbubble-based contrast ultrasound (US) agents, which are confined to the intravascular space, make impossible to compare the perfusion kinetics by the two methods.

Assessment of synovial vascularity with US techniques is well documented in literature. Indeed, color (D) and power Doppler (PD) US are used to evaluate synovial vascularity, but these modalities can detect only larger vessels with signals above the noise level or flow velocities above the threshold of the wall filter [23]. PDUS, revealing the movement of blood cells within a vessel, has been used to differentiate between hypervascular and fibrous pannus and to assess the therapeutic response to intra-articular treatment of synovitis of the knee joint. However, limitation of PDUS is represented by inadequacy to indicate increased vascularity or hyperaemia of the synovium, making often difficult interpretation of PD images [24–26].

Thus, contrast-enhanced US (CEUS) imaging, using second-generation contrast medium based on sulfur hexafluoride-filled microbubbles, and low mechanical index (MI) have overcome this limitations, improving the contrast and spatial resolution [27, 28]. In particular, second-generation contrast agents show a high and prolonged stability in the peripheral blood, being detected by gray scale harmonic imaging techniques, allowing a real-time analysis of all vascular phases and providing detailed information on tissue perfusion [27].

On the other hand, the superiority of gray-scale CEUS to detect synovial vascularity in comparison to PDUS has been assessed in RA patients, making possible the differentiation between active synovitis and inactive intra-articular thickening [28–31].

However, the value of the quantitative CEUS technique in the assessment of synovial tissue perfusion has not yet well established.

The purpose of this study is to assess the relationship of the CEUS evaluation with the histopathological features and immunohistochemical (IHC) quantitative estimation of microvascular proliferation on arthroscopic bioptic synovial samples of patients affected by sustained PsA. The ultimate goal is to improve the definition of the pathophysiological features of synovial angiogenesis, as the accuracy of these diagnostic tools for the objective assessment of synovial blood perfusion imaging in PsA.

Material and methods

Patients

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.

Clinical and demographic characteristics of the patients are reported in Table 1. All the patients participating in the study fulfilled the ClASsification criteria for Psoriatic ARthritis (CASPAR) [32]. The psoriasis area and severity index (PASI) was less than 10 in these patients.

We enrolled 32 consecutive PsA patients with refractory knee joint synovitis for study. All knees showed clinical evidence of joint effusion (positive bulge sign or ballottement of the patella, or both). All patients attend the Rheumatology Unit of Padova University Hospital. All had persistent active knee arthritis (characterized by pain, tenderness, and effusion). Mean duration of knee joint involvement was 6.9± 9.99 years (Table 1).

Gray scale and power Doppler US evaluation

Before contrast-enhanced study, gray scale and PD US examination were performed immediately after the clinical examination using an Esaote MyLab 25 Xvision ultrasound scanner (Esaote, Genoa, Italy) with a linear 7.5-MHz array transducer by experienced sonographers. Standardized anatomic guidelines of the scan in three recesses of the knee—superpatellar recess (SPR) and lateral and medial parapatellar recesses (LPPR and MPPR)—were used [33]. SPR was evaluated by scanning the zone between the prefemoral (posterior suprapatellar) fat pad and the upper margin of the femoral cartilage (supine position; knee joint extended; bicipes femoris at rest). At the level of LPPR and MPPR, the vertical edge along the lateral and medial margins of the knee cap (bicipes femoris contracted) was identified by scanning.

PDUS examination was conducted to assess flow signal in the pannus areas (more than 3 mm of synovial thickness) and was set for high sensitivity, with a low wall filter to allow detection of vessels with a low blood flow. The pulse repetition frequency was 750–1200 Hz, and medium persistence was used. The color gain was increased until background noise appeared and then reduced until noise was suppressed, thus ensuring maximum sensitivity.

The knee joint recess indicating the strongest vascularity in the synovial pannus on PDUS evaluation was chosen for examination by contrast-enhanced technique.

Table 1 Clinical and demographic characteristics of psoriatic arthritis patients included in the study

Clinical and demographic characteristics	Values
Patients (*n*)	32
PsA duration, years, mean±SD	6.32±4.75
Patients age, years, mean±SD	38.90±13.33
Knee joint synovitis duration	6.9±9.99 years
Female, *n* (%)	16 (47.05)
Systemic treatment at study entry	
DMARDs, *n* (%)	26 (81.25)
ETN, *n* (%)	6 (18.75)
Pdn[a], *n* (%)	6 (18.75)

PsA psoriatic arthritis, *DMARDs* disease-modifying antirheumatic drugs, *ETN* etanercept, *PDN* prednisolone

[a] Daily prednisolone dose ≤10 mg

Gray scale CEUS evaluation

Contrast-enhanced US examinations were performed by means of US apparatus (Esaote MyLab 25 Xvision ultrasound scanner; Esaote, Genoa, Italy), which was specifically designed for sonographic examinations with a contrast agent. A contrast agent composed of sulfur hexafluoride-filled microbubbles (SonoVue, Bracco International B.V., Amsterdam, Nederlands) was used.

A 7.5-MHz dedicated linear transducer was used in conjunction with a specific continuous mode contrast-enhanced harmonic imaging technology, "Contrast Tuned Imaging technology (CnTI)", which allows the transmission of the specific resonance frequency of sulfur hexafluoride-filled microbubbles, and the selective registration of harmonic frequencies. This allows a significant reduction of the insonation power with a reduction of the nonlinear harmonic behavior of the stationary tissues.

The acoustic pressure was set at 45 kPa in each patient, and the mechanical index (MI) was selected automatically by the sonography scanner in relation to beam focus depth.

All patients gave their informed consent for the use of contrast agent. A 4.8-mL bolus of contrast agent was injected into a peripheral vein and was followed by an injection of 10 mL of physiologic saline solution.

Immediately after the injections, the synovial tissue under examination was scanned in CnTI mode with a frame rate of 15 frames per second. The transducer was kept in a fixed position to highlight all the phases of enhancement. The beam focus was placed at the level of the synovial proliferation or immediately below it, and beam gain was set at the minimum level.

The apparatus in question affords the recording and filing of the images in digital format, and all the dynamic phases of the examination performed during 60 s were saved using this system.

Quantitative study

The digital recordings were sent from the sonography scanner to a PC and were then processed by means of a commercial software (Qontraxt, Amid and R&D, Bracco, Milan, Italy) allowing the generation of time-intensity curves of contrast

agent and the estimate of the perfusion parameters after setting of region of interest (ROIs).

The tissue perfusion evaluation of the pulse-inversion harmonic imaging contrast-enhanced ultrasound (CEUS) was performed by the automatic quantification assessment, using the video intensity data, in a simple mono-exponential model: SI(t))=PEAK*[1-e(-beta t)] (the simple exponential fitting curve assumes that the signal intensity is zero at the initial perfusion instant) (Figs. 1 and 2).

This software is able to analyze the signal intensity of each single pixel of each frame and thereby to generate a chromatic map that allows immediate evaluation of perfusion proprieties of the entire organ under examination or region of interest (ROIs), irrespective of shape or dimensions, as selected by the operator. In practice, a virtual image composed of a scale of primary colors varying from red (maximum signal intensity) to blue (minimum signal intensity) is obtained.

On the basis of this analysis, the software enables to obtain numeric values for each point in the region under examination, during a temporal sequence selected by the operator, as the final result. These values are correlated to the quantity of contrast medium that reached the examined area.

Contemporaneously, the system allows the generation of signal intensity-time curves in relation to the selected point or region and the estimate the quantitative perfusion parameters.

Since the microscopic and macroscopic heterogeneities in the synovial microcirculation in persistent RA, PsA, and SpA KJS, the level of enhancement is not uniform over the entire synovial area. For this reasons, the positions and dimensions

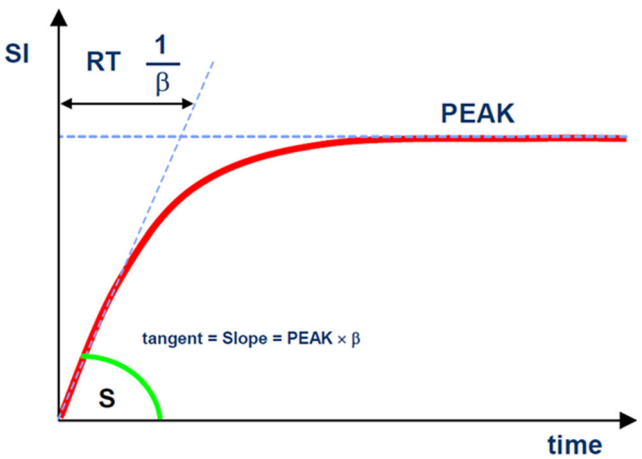

Fig. 1 Parameters of the pulse-inversion harmonic imaging [contrast-enhanced ultrasound (CEUS)*]. *PEAK* peak of the signal intensity (SI) which is reached asymptotically at the end of the perfusion process, proportional to regional blood volume (RBV); *RT* refilling time: the time when a line starting tangent to the curve at time t equal to zero reaches the PEAK value. Slope (S): inclination of the tangent in the origin. It is obtained by the product of PEAK and beta parameters; proportional to regional blood flow (RBF); β (beta): perfusion frequency. It is the inverse of the RT. *Qontraxt, Amid and R&D, Bracco, Milan, Italy.

of the ROIs at synovial pannus were chosen on the basis of gray scale contrast-enhanced study and were outlined including all hypervascular synovial area in the section of plane under examination.

Perfusional parameters including regional blood flow (RBF); peak (Peak) of the signal intensity (SI), which is reached asymptotically at the end of the perfusion process, and it is proportional to regional blood volume (RBV); beta (β) perfusion frequency; slope (S), which represents the inclination of the tangent in the origin, is obtained by the product of PEAK and β parameters, being proportional to the regional blood flow (RBF) and the refilling time (RT), the time when a line, starting tangent to the curve at time t equal to zero, reaches the PEAK value (RT=1/β), were analyzed.

Synovial histology and immunohistochemistry

Arthroscopic biopsies were carried out within 1 week after the examination in 32 active joints. Synovial tissue specimens were collected for biopsy during arthroscopy while the patients were under anesthesia. The specimens were taken from areas of intense synovial hyperemic proliferation. For each knee, synovial samples were always taken from the joint recess chosen for CEUS examination. The patients' serial biopsy samples were stored in paraformaldehyde and embedded in bloc in paraffin [17]. Synovial vessels were evaluated after immunostaining using CD31/PECAM-1 (clone JC70A) and CD105 (endoglin transmembrane homodimeric component of the transforming growth factor-ß type I receptor, as proliferation-antigen on angiogenic endothelial cells; SN6h) antibodies and mononuclear cell (MNC) infiltration using CD45/LCA (clone 2B11 and PD7-26) antibodies, labeling mononuclear cells, as monocytes, DC, and lymphocytes (DakoCytomation, Glostrup, Denmark).

CD45+, CD105+, and CD31+ expressions were measured by computer-assisted morphometric analysis (Image Proplus version 5), and a 2-mm two area was evaluated. The average value over four to six samples per knee joint was used for analysis.

Statistical analysis was carried out using an SpSS software (version 12). Spearman's rank correlation test was used for comparison between perfusional CEUS parameters and HIC index.

Results

Synovial histology and immunohistochemistry

Synovial histology showed slight increased layer lining thickness, perivascular lymphomonocyte cell infiltration, and microvascular remodeling. The vessels showed marked vessel

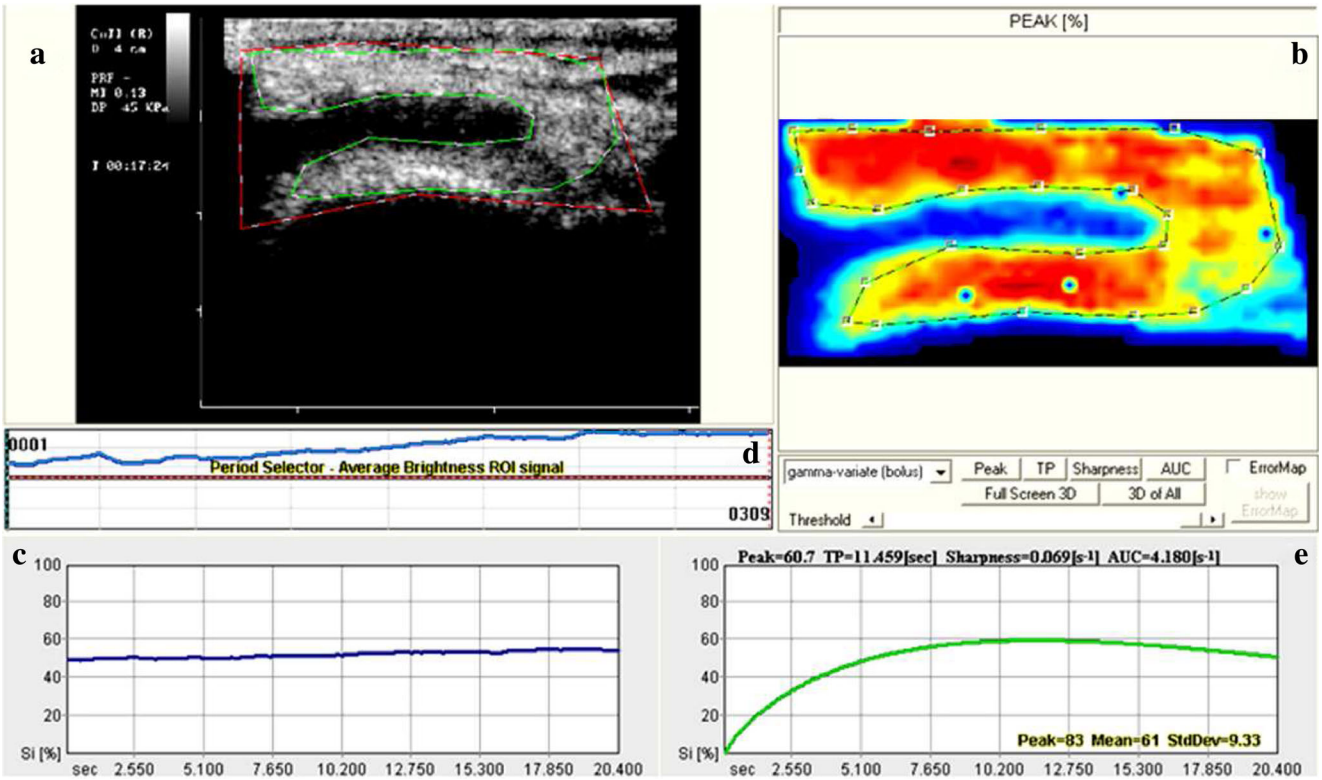

Fig. 2 **a–e** Main graphic window. After running the program, a graphical user interface (GUI) with the following Main Graphic Window will be displayed: **a** ROI window; **b** Parametric Map Window; **c** Time Selection Window; **d** the average brightness ROI signal; **e** ROI curve Window. **a** ROI window: after loading the clip, the user is prompted with a window where the external ROI must be defined. This external region will contain the area which will be analyzed on a pixel-by-pixel base by the software. **b** Parametric Map Window: parametric imaging is constituted of four synthetic colored maps that correspond to the four parameters of the fitted curve. The perfusion loop (i.e., the video intensity-time propagation due to the passage of the contrast agent in the tissue of interest) is visualized on the Parametric Map Window. Parametric maps are easy-to-interpret color images describing different aspects of the organ perfusion (see Fig. 1 for details). After processing, map for the parameter PEAK is shown as first on the right window of the GUI. The original values of the color-scale and the values obtained after moving the threshold are reported in the image representation. **c** Time Selection Window: the definition of the perfusion time period corresponds to the selection of the frames, within the loop, which enter into the analysis (commonly from the arrival of contrast media to the end of washout). The quantification assessment is performed on the frame sequence within the selected perfusion period. **d** Average brightness ROI signal: the average brightness variation over time will be displayed in the Time Selection Window. **e** ROI curve Window: the software performs the tissue perfusion evaluation using the video intensity data. The signal intensity (SI) extracted from the image during the contrast agent bolus passage or the refilling period is approximated by the standard parametric curve. The refilling curve is extracted for each pixel of the automaticallym aligned sequence of images

intimal medial thickening with significant reduction of the vascular lumen.

Immunohistochemistry for CD45 and CD31 highlighted inflammatory cell infiltration and neoangiogenesis (Figs. 3 and 4). The mean values of synovial immunohistochemistry for $CD45^+$ MNCs as for $CD31^+$ and $CD105^+$ endothelial cells are reported in Table 2

CEUS evaluation

The mean values of CEUS parameters are reported in Table 3. Significant correlations were found between the levels of CD31+ and either of CD105+ expression on synovial vessels (r 0.69; p=0.0000) or CD45+ MNCs (r 0.57; p=0.0007) in synovial tissue of PsA patients.

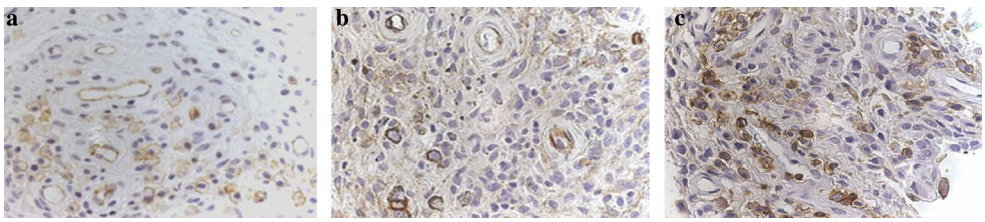

Fig. 3 **a–c** Immunohistochemical staining of CD31+ endothelial cells (**a**), CD105+ endothelial cells (**b**), and infiltrating CD45+ mononuclear cells (**c**) in synovial biopsy sample of the knee joints of patients with psoriatic arthritis included in the study

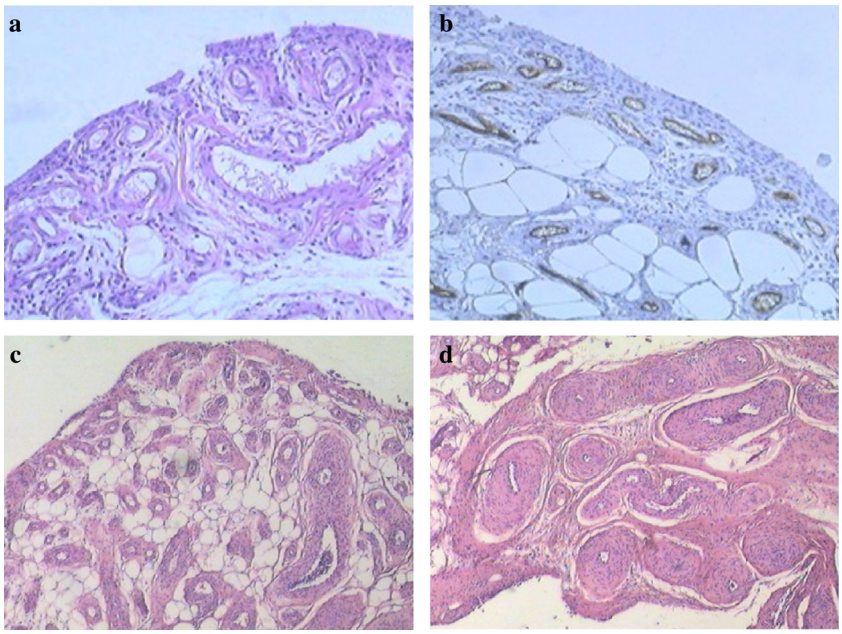

Fig. 4 **a–d** Synovial histology (hematoxylin and eosin staining of synovial tissue) in four representative patients with psoriatic arthritis included in the study

In the CEUS-enhanced area studied, which represents the section of the plane that contains variable blood vessels density within synovial tissue of very low volume flow, the corresponding time-intensity curves demonstrated a slow rise time. Among the synovial CEUS perfusion parameters, the RT, a function of both vascular volume and flow rate, showed a significant inverse relation either to synovial RBF, or PEAK, or S and β (Table 4).

The analysis of correlation between synovial perfusion and synovial vascularity showed a significant positive association between RT- and PEAK-CEUS parameters and the synovial tissue expression of CD31 or of CD105, respectively (Table 5).

Discussion

Dysregulation of angiogenesis, defined as blood vessels sprouting from pre-existing vessels, has shown to be crucial in the inflammatory arthritis such as in tumor growth [34, 35].

Table 2 Biological synovial markers

IHC positive cells in synovial tissue (cell/2-mm^2 area)[a]	Values
CD31	87.81±61.00
CD45	892.75±447.33
CD105	125.44±95.81

Data are expressed as mean±standard deviation (SD)

IHC immunohistochemistry

[a] Synovial biopsies obtained from 32 knees

The definition of the microvascular synovial perfusion hemodynamic, as the precise measure and delineation of the histopathological counterpart at single joint level, is of major importance for the development of reliable imaging methods [36–38]. Until now, the attempt to correlate the DUS or PDUS signal measures in arthritis, to synovial microvessels density by histopathology, has provided conflicting results. Recently, a cross-sectional study in hand joints of RA patients showed in line with previous findings [39] a significant association between DUS activity, determined maximum color fraction, and the extent of inflammation in the synovial biopsies, measured by immunohistochemical staining [40]. However, synovial pathology was also evident in 15 % of the biopsies taken from Doppler negative sites [40]. Discordant relationships between PD US imaging and histology were again reported in arthritis [41–43]. A positive correlation between the number of

Table 3 Values of contrast-enhanced ultrasound (CEUS) perfusional parameters derived by the evaluation of the 32 knee joints of psoriatic arthritis patients included in the study (Spearman's correlation test)

CEUS perfusional parameters	Values
RBV	0.67±6.16
Peak	31.69±10.33
β	0.60±0.57
S	8.83±6.93
RTs	5.63±2.31
RBF	5.73±3.33

Data are expressed as mean±standard deviation (SD)

CEUS contrast-enhanced ultrasound, *RBV* regional blood volume, *Peak* peak of the signal intensity, *β* perfusion frequency, *S* slope, *RT* refilling time, *RBF* regional blood flow

Table 4 Correlations between the different contrast-enhanced ultrasound parameters (CEUS) (Spearman's correlation test)

	RBV	PEAK	β	S	RBF
RBV	–	*r* 0.853 *p* 0.000	*r* 0.212 *p* n.s	*r* 0.156 *p* n.s	*r* 0.352 *p* 0.0478
PEAK	*p* n.s	–	*r* 0.207 *p* ns	*r* 0.102 *p* n.s	*r* 0.835 *p* 0.000
β	*p* n.s	–	–	*r* 0.857 *p* 0.000	*r* 0.740 *p* 0.000
S	*p* n.s	–	–	–	*r* 0.883 *p* 0.000
RT	*r* 0.200 *p* ns	*r* −0. 354 *p* −0.0469	*r* −0.435 *p* −0.0126	*r* −0.442 *p* −0.0113	*r* −0.5693 *p* −0.0007

RBV regional blood volume, *Peak* peak of the signal intensity, *β* perfusion frequency, *S* slope, *RT* refilling time, *RBF* regional blood flow

synovial vessels and amount of PD signal was found in RA and OA. However, the study examined only advanced stage of knee joints, requiring arthroplasty [41, 44]. Notably, the existence of Doppler signal in the synovium is not always specific for inflammation, since fibrosis and amount of fibrin could also give a positive PD [26]. To our knowledge, the only previous study using CEUS method showed the association between the percentage of area with acoustic enhancement and the immunohistology vessel density, but a limited number of patients with different arthritis were considered, as hemodynamic parameters of perfusion analysis were not reported in detail [45]. However, by using the gray scale CEUS in tumors,

Table 5 Correlations between contrast-enhanced ultrasound (CEUS) parameters and immunohistochemical (IHC) parameters CD31, CD105, and CD45 (Spearman's correlation test)

	CD31	CD105	CD45
RBV	*r* 0.020	*r* 0.22	*r* 0.23
	p ns	*p* ns	*p* ns
PEAK	*r* 0.04	*p* 0.0377	*r* 0.11
	p ns	*r* 0.369	*p* ns
β	*r* -0.026	*p* 0.0505	*r* 0.11
	p ns	*r* −0.348	*p* ns
S	*r* −0.16	*r* −0.11	*r* 0.12
	p ns	*p* ns	*p* ns
RT	*p* 0.0151	*r* −0.01	*r* 0.17
	r 0.425	*p* ns	*p* ns
RBF	*r* 0.260	*r* −0.06	*r* −0.18
	p ns	*p* ns	*p* ns
CD31	–	*p* 0.000	*p* 0.0007
	–	*r* 0.69	*r* 0.57

CD31 $CD31^+$ synovial vessel density, *CD105* $CD105^+$ synovial vessel density, *CD45* $CD45^+$ synovial mononuclear cell numbers, *RBV* regional blood volume, *Peak* peak of the signal intensity, *β* perfusion frequency, *S* slope, *RT* refilling time, *RBF* regional blood flow

the analysis of contrast enhancement kinetics the blood flow was highly correlated to microvascular density. It may derive from the characteristic perfusion kinetics of malignant tumors, with a tendency toward faster and stronger enhancement and greater perfusion flow [46, 47]. Likewise, the contrast enhancement perfusion patterns were associated to the morphologic features of vessel histology [48].

The first important finding of this study comparing perfusion kinetics using CEUS technique with the detection of IHC synovial vascularity in PsA joints was the demonstration of a direct correlation between contrast-refilling time and the CD31+ microvessel density. Our finding suggests that the bulk of proliferated vascular network, by increasing the resistance to synovial vessel flow, might determine the prolongation of the RT. Among the potential factors which may affect the synovial refilling time, the vessel intrinsic factors, as tortuosity of the microvasculature, reduced vessel diameter, or capillary obliteration [38], could be involved. A reduction in synovial blood flow during the progression of arthritis was noted after induction of experimental arthritis [49], as a reduced blood volume fraction of synovial capillaries has been observed in RA [2, 36].

The histopathological examination of the synovial biopsies of knee joints affected by sustained PsA showed striking microvascular changes, characterized by marked vessel wall proliferation, basement membrane thickening, and sclerosis, with reduction of the size of vascular lumen. These microvascular changes resemble typical morphology of the synovial vessel, corresponding in turn to that described in the seminal work of Espinoza, mainly involving small and medium size arterioles [1]. The reduction of the lumen of synovial vessels may be a relevant factor in the perfusion kinetics obtained by CEUS, since the vessel diameter represents the dominant parameter in flow regulation in a single vessel [38].

In line with the CEUS perfusion findings of the study, when PsA knees were compared to RA using DCE-MRI analysis, a significantly lower rate of late enhancement (after 15′) was detected in the joints of PsA, at a time when vessel intrinsic factors seemed to play a predominant role in delayed synovial washout rate [50], and in PsA only, DCE-MRI contrast enhancement was unrelated to the inflammation parameters [50, 51].

The next new finding of the study was the positive association of PEAK of contrast intensity, (proportional to regional synovial blood volume) with the CD105+ microvessel density, representing the new formed capillary network [52], which is rapidly growing in the scattered areas of more active neovascularity in PsA [7].

Validation of CEUS to quantify synovial inflammation has been reported in several studies, allowing the improved detection of intra-articular microvascularity compared with the traditional enhanced PDUS [28], since it may overcome the limitation of the PDUS methods of detection of only the fast blood flow of large vessels. In patients affected by PsA and

SpA, the CEUS technique was shown also to increase the detection of synovial hypervascularity in knees, compared with the unenhanced PD method [29], investigating active sacroiliitis [31] and enthesitis [53].

Among the limitations of gray scale CEUS techniques in arthritis assessment, there are the micro-invasiveness of the method, the cost and time expensive technique, the only one joint, which can be once examined, and the assessment of perfusion in the single plane or section. Furthermore, diagnostic value and reproducibility in monitoring arthritis treatment are not well established [49].

Recent studies indicate that subclinical enthesitis and synovitis, mostly in wrist and knee joints, are very common either in psoriasis and in early PsA disease [54, 55], being linked by a particular "vascular phenotype" [56, 57], characterized by high PD scores [57]. Further, the frequency of extra-articular PD signal at metacarpophalangeal joints allowed to differentiate PsA from RA [58], as joint and tendon-related PD scores were higher in patients with active versus inactive PsA disease [59]. All the data suggest the requirement of a reliable, quantitative measure of synovial perfusion for monitoring the PsA treatment, as has been proven by the use of CEUS method in the follow-up of the angiogenesis inhibitors in tumors [60]. In this view, as the decrease of PD flow of the capillary network confined to the outer synovial surface layer, was reported to precede the change in gray scale synovial thickening [29], CEUS-PEAK might represent a useful tool for the detection of early changes of synovial blood perfusion.

In conclusion, the study demonstrated the association of the CEUS perfusion kinetics with the histopathological quantitative and morphologic estimation of synovial microvascular proliferation, providing more insight on the relationship between perfusion kinetics and synovium vascular proliferation process in PsA, also suggesting that a contrast-assisted ultrasound imaging represents a reliable tool for the estimate of the synovial hypervascularity in arthritis.

Conflict of interest The authors declare that they have no conflicts of interest.

References

1. Espinoza LR, Vasey FB, Espinoza CG, Bocanegra TS, Germain BF (1982) Vascular changes in psoriatic synovium. A light and electron microscopic study. Arthritis Rheum 25:677–84
2. Stevens CR, Blake DR, Merry P, Revell PA, Levick JR (1991) A comparative study by morphometry of the microvasculature in normal and rheumatoid synovium. Arthritis Rheum 34:1508–13
3. Ceponis A, Konttinen YT, Imai S, Tamulaitiene M, Li TF, Xu JW, Hietanen J, Santavirta S, Fassbender HG (1998) Synovial lining, endothelial and inflammatory mononuclear cell proliferation in synovial membranes in psoriatic and reactive arthritis: a comparative quantitative morphometric study. Br J Rheumatol 37:170–8
4. Baeten D, Demetter P, Cuvelier C, van den Bosch F, Kruithof E, van Damme N, Verbruggen G, Mielants H, Veys EM, de Keyser F (2000) Comparative study of the synovial histology in rheumatoid arthritis, spondyloarthropathy, and osteoarthritis: influence of disease duration and activity. Ann Rheum Dis 59:945–53
5. Reece RJ, Canete D, Parsons WJ, Emery P, Veale DJ (1999) Distinct vascular patterns of early synovitis in psoriatic, reactive and rheumatoid arthritis. Arthritis Rheum 42:1481–4
6. Fraser A, Fearon U, Reece R, Emery P, Veale DJ (2001) Matrix metalloproteinase 9, apoptosis, and vascular morphology in early arthritis. Arthritis Rheum 44:2024–8
7. Fiocco U, Cozzi L, Chieco-Bianchi F, Rigon C, Vezzù M, Favero E, Ferro F, Sfriso P, Rubaltelli L, Nardacchione R, Todesco S (2001) Vascular changes in psoriatic knee joint synovitis. J Rheumatol 28: 2480–6
8. Cañete JD, Rodríguez JR, Salvador G, Gómez-Centeno A, Muñoz-Gómez J, Sanmartí R (2003) Diagnostic usefullness of synovial vascular morphology in chronic arthritis. A systematic survey of 100 cases. Semin Arthritis Rheum 32:378–387
9. McGonagle D, Lories RJ, Tan AL, Benjamin M (2007) The concept of a "synovioentheseal complex" and its implications for understanding joint inflammation and damage in psoriatic arthritis and beyond. Arthritis Rheum 56:2482–91
10. Linblad S, Hedfors E (1985) Intraarticular variation in synovitis. Local macroscopic and microscopic signs of inflammatory activity are significantly correlated. Arthritis Rheum 28:977–86
11. Veale D, Yanni G, Rogers S, Barnes L, Bresnihan B, Fitzgerald O (1993) Reduced synovial membrane macrophage numbers, ELAM-1 expression, and lining layer hyperplasia in psoriatic arthritis as compared with rheumatoid arthritis. Arthritis Rheum 36:893–900
12. van Kuijk AW, Reinders-Blankert P, Smeets TJ, Dijkmans BA, Tak PP (2006) Detailed analysis of the cell infiltrate and the expression of mediators of synovial inflammation and joint destruction in the synovium of patients with psoriatic arthritis: implications for treatment. Ann Rheum Dis 65:1551–7
13. Cañete JD, Pablos JL, Sanmartí R, Mallofré C, Marsal S, Maymó J, Gratacós J, Mezquita J, Mezquita C, Cid MC (2004) Antiangiogenic effects of anti-tumor necrosis factor alpha therapy with infliximab in psoriatic arthritis. Arthritis Rheum 50:1636–41
14. Paavonen K, Mandelin J, Partanen T, Jussila L, Li TF, Ristimaki A, Alitalo K, Konttinen YT (2002) Vascular endothelial growth factors C and D and their VEGFR-2 and 3 receptors in blood and lymphatic vessels in healthy and arthritic synovium. J Rheumatol 29:39–45
15. Fearon U, Griosios K, Fraser A, Reece R, Emery P, Jones PF, Veale DJ (2003) Angiopoietins, growth factors, and vascular morphology in early arthritis. J Rheumatol 30:260–8
16. Krausz S, Garcia S, Ambarus CA, de Launay D, Foster M, Naiman B, Iverson W, Connor JR, Sleeman MA, Coyle AJ, Hamann J, Baeten D, Tak PP, Reedquist KA (2012) Angiopoietin-2 promotes inflammatory activation of human macrophages and is essential for murine experimental arthritis. Ann Rheum Dis 71:1402–10
17. Fiocco U, Sfriso P, Oliviero F, Roux-Lombard P, Scagliori E, Cozzi L, Lunardi F, Calabrese F, Vezzu' M, Dainese S, Molena B, Scanu A, Nardacchione R, Rubaltelli L, Dayer JM, Punzi L (2010) Synovial effusion and synovial fluid biomarkers in psoriatic arthritis to assess intraarticular tumor necrosis factor-a blockade in the knee joint. Arthritis Res Ther 12:R148
18. Gaffney K, Cookson J, Blades S, Coumbe A, Blake D (1998) Quantitative assessment of the rheumatoid synovial microvascular bed by gadolinium-DTPA enhanced magnetic resonance imaging. Ann Rheum Dis 57:152–7
19. Ostergaard M, Stoltenberg M, Løvgreen-Nielsen P, Volck B, Sonne-Holm S, Lorenzen I (1998) Quantification of synovistis by MRI: correlation between dynamic and static gadolinium-enhanced magnetic resonance imaging and microscopic and macroscopic signs of synovial inflammation. Magn Reson Imaging 16:743–54

20. Newman CJ, Laing TJ, McCarthy JS, Adler RS (1996) Power Doppler sonography of synovitis: assessment of therapeutic response. Preliminary observations. Radiology 198:582–4
21. van der Leij C, van de Sande MG, Lavini C, Tak PP, Maas M (2009) Rheumatoid synovial inflammation: pixel-by-pixel dynamic contrast-enhanced MR imaging time-intensity curve shape analysis–a feasibility study. Radiology 253:234–40
22. van de Sande MG, van der Leij C, Lavini C, Wijbrandts CA, Maas M, Tak PP (2012) Characteristics of synovial inflammation in early arthritis analysed by pixel-by-pixel time-intensity curve shape analysis. Rheumatology (Oxford) 51:1240–5
23. Torp-Pedersen ST, Terslev L (2008) Setting and artefacts relevant in colour/power Doppler ultrasound in rheumatology. Ann Rheum Dis 67:143–9
24. Szkudlarek M, Court-Payen M, Strandberg C, Klarund M, Klausen T, Ostergaard M (2001) Power Doppler ultrasonography for assessment of synovitis in the metacarpophalangeal joints of patients with rheumatoid arthritis. Arthritis Rheum 44:2018–23
25. Carotti M, Salaffi F, Manganelli P, Salera D, Simonetti B, Grassi W (2002) Power Doppler sonography in the assessment of synovial tissue of the knee joint in rheumatoid arthritis: a preliminary experience. Ann Rheum Dis 61:877–82
26. Koski JM (2012) Doppler imaging and histology of the synovium. J Rheumatol 39:452–3
27. Albrecht T, Urbank A, Mahler M, Bauer A, Doré CJ, Blomley MJ, Cosgrove DO, Schlief R (1998) Prolongation and optimization of Doppler enhancement with a microbubble US contrast agent by using continuous infusion: preliminary experience. Radiology 207:339–47
28. Klauser A, Frauscher F, Schirmer M, Halpern E, Pallwein L, Herold M, Helweg G, ZurNedden D (2002) The value of contrast-enhanced color Doppler ultrasound in the detection of vascularization of finger joints in patients with rheumatoid arthritis. Arthritis Rheum 46:647–53
29. Fiocco U, Ferro F, Cozzi L, Vezzù M, Sfriso P, Checchetto C, Bianchi FC, Nardacchione R, Piccoli A, Todesco S, Rubaltelli L (2003) Contrast medium in power Doppler ultrasound for assessment of synovial vascularity: comparison with arthroscopy. J Rheumatol 30: 2170–6
30. Terslev L, Torp-Pedersen S, Bang N, Koenig MJ, Nielsen MB, Bliddal H (2005) Doppler ultrasound findings in healthy wrists and finger joints before and after use of two different contrast agents. Ann Rheum Dis 64:824–7
31. Klauser AS, de Zordo T, Bellmann-Weiler R, Feuchtner GM, Sailer-Höck M, Sögner P, Gruber J (2009) Feasibility of second-generation ultrasound contrast media in the detection of active sacroiliitis. Arthritis Rheum 61:909–16
32. Taylor W, Gladman D, Helliwell P, Marchesoni A, Mease P, Mielants H, CASPAR Study Group (2006) Classification criteria for psoriatic arthritis: development of new criteria from a large international study. Arthritis Rheum 54:2665–73
33. Fiocco U, Ferro F, Vezzù M, Cozzi L, Checchetto C, Sfriso P, Botsios C, Ciprian L, Armellin G, Nardacchione R, Piccoli A, Todesco S, Rubaltelli L (2005) Rheumatoid and psoriatic knee synovitis: clinical, grey scale, and power Doppler ultrasound assessment of the response to etanercept. Ann Rheum Dis 64:899–905
34. Haywood L, Walsh DA (2001) Vasculature of the normal and arthritic synovial joint. Histol Histopathol 16:277–84
35. Folkman J (1971) Tumor angiogenesis: therapeutic implications. N Engl J Med 285:1182–1186
36. Levick JR (1990) Hypoxia and acidosis in chronic inflammatory arthritis; relation to vascular supply and dynamic effusion pressure. J Rheumatol 17:579–582
37. Kurikawa Y, Ahlqvist J (1998) Joint fluid hydrostatic pressures that empty synovial capillaries of red cells have a wide range and correlate with pressures emptying arterioles. J Rheumatol 25:1364–8
38. Jain RK (1988) Determinants of tumor blood flow: a review. Cancer Res 48:2641–2658
39. Takase K, Ohno S, Takeno M, Hama M, Kirino Y, Ihata A, Ideguchi H, Mochida Y, Tateishi U, Shizukuishi K, Nagashima Y, Aoki I, Ishigatsubo Y (2012) Simultaneous evaluation of long-lasting knee synovitis in patients undergoing arthroplasty by power Doppler ultrasonography and contrast-enhanced MRI in comparison with histopathology. Clin Exp Rheumatol 30:85–92
40. Andersen M, Ellegaard K, Hebsgaard JB, Christensen R, Torp-Pedersen S, Kvist PH, Søe N, Rømer J, Vendel N, Bartels EM, Danneskiold-Samsøe B, Bliddal H (2014) Ultrasound colour Doppler is associated with synovial pathology in biopsies from hand joints in rheumatoid arthritis patients: a cross-sectional study. Ann Rheum Dis 73:678–83
41. Koski J, Saarakkala S, Helle M, Hakulinen U, Heikkinen JO, Hermunen H (2006) Power Doppler US and synovitis: correlating ultrasound imaging with histopathological findings and evaluating the performance of US equipment. Ann Rheum Dis 65:1590–5
42. Walther M, Harms H, Krenn V, Radke S, Faehndrich T-P, Gohlke F (2001) Correlation of power Doppler sonography with vascularity of the synovial tissue of the knee joint in patients with osteoarthritis and rheumatoid arthritis. Arthritis Rheum 44:331–338
43. Motomura H, Matsushita I, Seki E, Kimura T (2013) Correlation of power doppler ultrasonographic findings with site-matched histopathology of the synovial tissue. Ann Rheum Dis 72(Suppl3):546
44. Walther M, Harms H, Krenn V, Radke S, Kirschner S, Gohlke F (2002) Synovial tissue of the hip at power Doppler US: correlation between vascularity and power Doppler US signal. Radiology 225: 225–31
45. Kaiser MJ, Hauzeur JP, Blacher S, Foidart JM, Deprez M, Rossknecht A, Malaise MG (2009) Contrast-enhanced coded phase-inversion harmonic sonography of knee synovitis correlates with histological vessel density: 2 automated digital quantifications. J Rheumatol 36:391–400
46. Du J, Li FH, Fang H, Xia JG, Zhu CX (2008) Correlation of real-time gray scale contrast-enhanced ultrasonography with microvessel density and vascular endothelial growth factor expression for assessment of angiogenesis in breast lesions. J Ultrasound Med 27:821–31
47. Dunnwald LK, Gralow JR, Ellis GK, Livingston RB, Linden HM, Specht JM, Doot RK, Lawton TJ, Barlow WE, Kurland BF, Schubert EK, Mankoff DA (2008) Tumor metabolism and blood flow changes by positron emission tomography: relation to survival in patients treated with neoadjuvant chemotherapy for locally advanced breast cancer. J Clin Oncol 26:4449–57
48. Xiao JD, Zhu WH, Shen SR (2010) Evaluation of hepatocellular carcinoma using contrast-enhanced ultrasonography: correlation with microvessel morphology. Hepatobiliary Pancreat Dis Int 9:605–10
49. Rednic N, Tamas MM, Rednic S (2011) Contrast-enhanced ultrasonography in inflammatory arthritis. Med Ultrason 13:220–7
50. Schwenzer NF, Kötter I, Henes JC, Schraml C, Fritz J, Claussen CD, Horger M (2010) The role of dynamic contrast-enhanced MRI in the differential diagnosis of psoriatic and rheumatoid arthritis. AJR Am J Roentgenol 194:715–20
51. Cimmino MA, Parodi M, Innocenti S, Succio G, Banderali S, Silvestri E, Garlaschi G (2005) Dynamic magnetic resonance of the wrist in psoriatic arthritis reveals imaging patterns similar to those of rheumatoid arthritis. Arthritis Res Ther 7:R725–31
52. Lebrin F, Goumans MJ, Jonker L, Carvalho RL, Valdimarsdottir G, Thorikay M, Mummery C, Arthur HM, ten Dijke P (2004) Endoglin promotes endothelial cell proliferation and TGF-beta/ALK1 signal transduction. EMBO J 23:4018–28
53. Mouterde G, Aegerter P, Correas JM, Breban M, D'Agostino MA (2014) Value of contrast-enhanced ultrasonography for the detection and quantification of enthesitis vascularization in patients with spondyloarthritis. Arthritis Care Res (Hoboken) 66:131–8

54. Naredo E, Möller I, de Miguel E, Batlle-Gualda E, Acebes C, Brito E, Mayordomo L, Moragues C, Uson J, de Agustín JJ, Martínez A, Rejón E, Rodriguez A, Daudén E, Ultrasound School of the Spanish Society of Rheumatology and Spanish ECO-APs Group (2011) High prevalence of ultrasonographic synovitis and enthesopathy in patients with psoriasis without psoriatic arthritis: a prospective case-control study. Rheumatology (Oxford) 50:1838–48
55. Freeston JE, Coates LC, Nam JL, Moverley AR, Hensor EM, Wakefield RJ, Emery P, Helliwell PS, Conaghan PG (2014) Is there subclinical synovitis in early psoriatic arthritis? A clinical comparison with gray-scale and power Doppler ultrasound. Arthritis Care Res (Hoboken) 66:432–9
56. McGonagle D, Aydin SZ, Tan AL (2012) The synovio-entheseal complex and its role in tendon and capsular associated inflammation. J Rheumatol Suppl 89:11–4
57. Aydin SZ, Ash ZR, Tinazzi I, Castillo-Gallego C, Kwok C, Wilson C, Goodfield M, Gisondi P, Tan AL, Marzo-Ortega H, Emery P, Wakefield RJ, McGonagle DG (2013) The link between enthesitis and arthritis in psoriatic arthritis: a switch to a vascular phenotype at insertions may play a role in arthritis development. Ann Rheum Dis 72:992–5
58. Gutierrez M, Filippucci E, Salaffi F, di Geso L, Grassi W (2011) Differential diagnosis between rheumatoid arthritis and psoriatic arthritis: the value of ultrasound findings at metacarpophalangeal joints level. Ann Rheum Dis 70:1111–4
59. Husic R, Gretler J, Felber A, Graninger WB, Duftner C, Hermann J, Dejaco C (2014) Disparity between ultrasound and clinical findings in psoriatic arthritis. Ann Rheum Dis 73:1529–36
60. McCarville MB, Streck CJ, Dickson PV, Li CS, Nathwani AC, Davidoff AM (2006) Angiogenesis inhibitors in a murine neuroblastoma model: quantitative assessment of intratumoral blood flow with contrast-enhanced gray-scale US. Radiology 240:73–81