Study of the association between nailfold capillaroscopic changes and serum level of interleukin-17 in rheumatoid: a clue for emerging vasculitis
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Objectives
The aim of this work was to study nailfold capillaroscopic (NC) abnormalities and serum interleukin-17 (IL-17) level among rheumatoid arthritis (RA) patients and to find whether IL-17 is causally involved in the changes in the capillary vascular bed, such as autoimmune prevasculitic changes.

Patients and methods
The study was conducted on a group of RA Egyptian patients (n=40) who were diagnosed as having RA based on ACR criteria. Those 40 patients were further divided into two groups. Group 1 included RA patients with clinical signs of skin vasculitis and NC changes (n=6). Group 2 included RA patients with no clinical signs of skin vasculitis and no NC changes (n=34). All patients were subjected to demographic data collection, clinical examination, disease activity score 28 calculation, laboratory measurement (including erythrocyte sedimentation rate, C-reactive protein, rheumatoid factor, antinuclear antibody, antineutrophil cytoplasmic antibody, anti-cyclic citrullinated peptide, and IL-17) and NC examination. In addition, group 1 (n=6) was further subjected to electrophysiological evaluation using peripheral nerve conduction studies to determine the effect of vasculitis on the peripheral nerves.

Results
IL-17 level and NC changes showed a significant association in RA vasculitis patients.

Conclusion
Elevated levels of serum IL-17 and characteristic NC changes raise their importance in the detection of preclinical rheumatoid vasculitis.

Keywords:
interleukin-17, nailfold capillaroscopy, rheumatoid vasculitis

Introduction
Rheumatoid vasculitis (RV) is the most serious systemic disease manifestation of rheumatoid arthritis (RA), wherein it manifests almost exclusively in RA patients with rheumatoid autoantibodies and often occurs in the context of other extra-articular manifestations. RV is defined as a clinicopathological manifestation of RA characterized by tissue damage or ischemia verified pathologically by vasculitis [1,2].

The prevalence of RV was estimated at less than 1–5%, whereas autopsy studies have reported 15–31% [3,4].

Three histological patterns of vasculitis may be seen:

(1) A necrotizing leukocytoclastic vasculitis of dermal venules is seen in patients with palpable purpura, hemorrhagic bullae, maculopapular erythema, and erythema elevatum diutinum [5].

(2) An acute or healing arteritis of dermal subcutaneous vessels similar to that seen in polyarteritis nodosa is seen in patients with subcutaneous nodules, livedo reticularis, and ulcers [6,7].

(3) Other histological patterns that can be seen include folliculocentric microabscess formation resembling dermatitis herpetiform and granulomatous vasculitis composed mainly of lymphocytes and histocytes [8].

The postulated mechanisms of vessel wall destruction with RV include the following: [9]

(1) Autoantibody targeting of the vessel [10].
(2) Incidental inflammation due to deposition of immune complexes [11,12].
(3) Collateral damage due to a local antigen-driven cellular immune response [13].

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RV may involve virtually any organ of the body, but the most common sites of involvement are the skin and peripheral nerves, as in many series the skin or peripheral nerves are involved in more than 80% of RV patients. Major organ system involvement of the heart, bowel, or kidney is much less common but can lead to significant morbidity and mortality, including myocardial infarction, bowel ischemia, and renal failure; although central nervous system involvement is rare, many case reports describe its occurrence [14,15].

Interleukin-17 (IL-17) or IL-17 A is a member of a group of cytokines called IL-17 family (including IL-17 B, IL-17C, IL-17 D, IL-17 E, and IL-17 F). Among all members, the biological functions and regulations of IL-17 A and IL-17 F are best understood [16,17]. Specialized T cells, called T-helper 17 cells, are the major sources of IL-17 A and IL-17 F in many types of adaptive immunity; however, recently, other contributors to IL-17 A and IL-17-F production, mainly in the innate arm of the immune system, were identified [18]. Although crucial in protecting the host from invasion by many types of pathogens, dysregulated IL-17 production can result in excessive proinflammatory cytokine expression and chronic inflammation, which lead to tissue damage and autoimmunity [19,20].

IL-17 has a role in chronic vascular inflammation of atherosclerosis and possibly hypertensive vascular changes, whereas in acute inflammation IL-17 is elevated and may be causally involved in the autoimmune vasculitides [21–25].

Nailfold capillaroscopy (NC) was used during the beginning of the 20th century, to show in detail the abnormalities that characterize the involvement of microvasculature during Raynaud’s phenomenon in systemic sclerosis [26]. NC represents the best method to analyze microvascular abnormalities in autoimmune rheumatic diseases [27].

In addition, capillaroscopic changes have been observed in systemic sclerosis, systemic lupus erythematosus, antiphospholipid syndrome, and Sjögren’s syndrome, but still further epidemiological and clinical studies are needed to better standardize the NC patterns [28].

**Aim**
The aim of this study was to study NC abnormalities and serum IL-17 level among RA patients and to find whether IL-17 is causally involved in the changes in the capillary vascular bed, such as autoimmune vasculitic changes.

**Patients and methods**
The current study was conducted on 40 RA patients who were diagnosed according to the current 2010 ACR-EULAR criteria for diagnosis of RA. All patients gave their formal consent. The protocol was approved the Ethical committee of the Alexandria University.

Exclusion criteria included any disease or medication causing vasospasm, any other rheumatic disease, diabetes mellitus, and having hepatitis B or C viruses.

All patients were subjected to demographic data collection, historical data, clinical examination, calculation of disease activity score 28 (DAS 28), laboratory measurement [including erythrocyte sedimentation rate, C-reactive protein (CRP), rheumatoid factor (RF), anti-cyclic citrullinated peptide (anti-CCP), antinuclear antibody (ANA), antineutrophil cytoplasmic antibody (ANCA) ‘both types P and C’, and IL-17], and NC, wherein the following capillaroscopic parameters were evaluated using an ophthalmoscope: distribution, presence of dilatation, presence of avascular areas, hemorrhages, and neoangiogenesis.

Thereafter, the 40 patients were further divided into two groups based on the presence or absence of NC changes: RA patients with NC changes (group 1; n=6) and RA patients with no NC changes (group 2; n=34).

Thereafter, the six patients included in group 1 were further subjected to electrophysiological studies (in upper limbs: the median nerve and the ulnar nerve; and in the lower limb: the sural nerve and the posterior tibial nerve).

**Results**
The data collected from patients and controls were tabulated and statistically analyzed to study NC abnormalities and serum IL-17 pattern among RA patients with suspected vasculitis by positive NC changes and those with negative capillaroscopy.

The NC changes that were found in group 1 (n=6) were tabulated.

RA patients with NC changes (group 1) showed clinical evidence of skin vasculitis, which in our studied patients were as follows: three patients suffered from purpuric rash, two patients suffered from small ulcers on the feet
and big toe, and one patient suffered from livedo reticularis. All patients included in group 2 with no NC changes showed no clinical manifestations of skin vasculitis.

Patients of group 1 (n=6) showed the same electrophysiological findings and were as follows: absent sural nerve and normal conduction of other nerves.

DAS 28 showed a significant difference between RA patients with no NC changes (n=34) and RA patients with NC changes (n=6) (Table 1 and Fig. 1).

Twenty-one patients showed moderate disease activity (DAS 28>3.2 and ≤5.1) and the remaining 19 patients showed severe disease activity (DAS 28>5.1). The six RA patients with vasculitic changes were all found to show severe activity (DAS 28>5.1).

Erythrocyte sedimentation rate and anti-CCP showed no significant difference between RA patients with no NC changes (n=34) and RA patients with NC changes (n=6). In contrast, CRP and RF showed a significant difference between those two groups (P=0.010 and 0.001, respectively) (Table 2 and Figs 2 and 3).

ANA was compared between RA patients with no NC changes (n=34) and RA patients with NC changes (n=6), and it showed no significant difference (Table 3).

ANCA (both types showed the same results) showed a significant difference between RA patients with no NC changes (n=34) and RA patients with NC changes (n=6) (P=0.021) (Table 3 and Fig. 4).

When IL-17 serum level was compared between RA patients with NC changes (n=6) and RA patients with no NC findings (n=34), a significant difference was found (P<0.001) (Tables 4 and 5 and Fig. 5).

Discussion
The present study included 40 RA patients, of whom only six patients were diagnosed clinically as having RV. This is in agreement with the results of previously published data by Puechal et al. [4], which estimated the prevalence of RV at less than 1–5% in patients and 15–30% in autopsy. Similarly, Loricera et al. [29] reported a low incidence of RV.

In addition, the current study estimated that the incidence of NC changes is the same as the incidence of vasculitis in the studied patients, which shows that NC changes occur in parallel with clinical vasculitic changes. Similarly, Souza and Kayser [30] concluded that NC changes were associated with vascular disorders.

In contrast, Hachulla et al. [31] concluded the opposite, as the results showed that capillaroscopy when compared between RA patients with and those without vasculitis showed no difference, and this was attributed to the fact that the regular capillaroscopy was not accurate in detecting the vascular changes in the nailbed of RV patients.

Table 1 Nailfold capillaroscopic changes

| Patient 1 | Patient 2 | Patient 3 | Patient 4 | Patient 5 | Patient 6 |
|-----------|-----------|-----------|-----------|-----------|-----------|
| Loops dilated, areas of hemorrhage, and avascular areas | Loops dilated, areas of hemorrhage, and avascular areas | Dilated loops, and areas of hemorrhage. | Dilated loops, hemorrhage, and avascular areas | Dilated loops | Dilated loops |

Figure 1

Box plot showing disease activity score 28 (DAS 28) range in rheumatoid arthritis patients with and without nailfold capillaroscopic changes.

Table 2 Disease activity score 28 in rheumatoid arthritis with nailfold capillaroscopic changes and rheumatoid arthritis patients with no nailfold capillaroscopic changes

| RA patients with NC changes (group 1) (N=6) (15%) | RA patients without NC changes (group 2) (N=34) (85%) |  
|--------------------------------------------------|--------------------------------------------------|---|
| DAS 28 | Median (min.–max.) |  
| 6.41 (5.94–7.41) | 4.83 (3.29–7.88) | 0.001* |

DAS, disease activity score; min., minimum; max, maximum; NC, nailfold capillaroscopic; RA, rheumatoid arthritis. *P≤0.05 are significant.
This study showed a significant difference in DAS 28 between RA patients and RV patients, and concluded that patients with higher DAS 28 score were associated with vasculitic changes. This means that vasculitis in RA occurs in the disease with high activity as scored with DAS 28 in the studied patients.

Similarly, Shanmugan et al. [32] calculated DAS 28 in RA patients before and after the appearance of nailfold capillaroscopic changes.
vasculitic ulcer, and it was concluded that there were significant increases in DAS 28 score, which implied a significant difference. In contrast, Mederos et al. [33] concluded that there were discrepancies in data from DAS 28 and recommended to define better score with better cutoff.

In the current study, anti-CCP had no significant difference between RA and RV patients. This implies that anti-CCP is rather a diagnostic than a follow-up tool and its level does not reflect associated vascular abnormalities. In agreement, Wiik et al. [34] concluded that although anti-CCP is considered an important marker of progressive erosive disease and extra-articular manifestations such as vasculitis, this was hampered by doubt about a positive versus negative reaction in several assays. Korkmaz et al. [35] also concluded that anti-CCP does not seem to have much linkage with extra-articular manifestations of RA.

In contrast, Turesson et al. [36] concluded that anti-CCP levels tend to be higher in patients with RA with vasculitis than in those without vasculitis.

We showed reports that RF showed a significant difference between RA and RV patients, and it was higher in RV patients ($P=0.001$). This means that RF is highly linked and associated with vascular activity.

Similarly, Khasnis and Langford [37] and Turesson and Matterson [38] concluded that RF level is higher in vasculitis.

In contrast, Tourin et al. [39] proved that RA-associated vasculitis is associated with low titer-positive RF.

The current study illustrated that there was a significant difference in CRP between RA and RV patients, and CRP was higher in the RV group ($P=0.010$). This reflects the concluded association between CRP and vasculitic changes. Similarly, Suresh [40] concluded the association of high levels of CRP with RV. Moreover, Kobak et al. [41] showed that there was a significant difference in CRP between RA and RV patients.

This is contradictory to that reported by Senolt et al. [42], who showed that CRP can be normal in RA with or without extra-articular manifestations.

The current study concluded that there was no significant difference in ANA between RA and RV patients. This means that ANA is not associated with vasculitic changes in our studied patients.

In agreement, De Souza et al. [43] proved that ANA can be negative, with no significant difference in RV. In contrast, Voskuyl et al. [44] proved that ANA is positive and had significant difference in RV patients.

The current study concluded that ANCA showed a significant difference between RV patients and RA patients ($P=0.021$). This means that ANCA is associated with vasculitic changes occurring on top of RA in our studied patients.

Similarly, Voskuyl et al. [44] concluded that there is a significant difference in ANCA between RA and RV patients.

In contrast, Puechal [45] and Suresh [40] excluded the possibility of the presence of a significant difference between RA and RV patients.

The current study concluded that there was a significant difference in IL-17 between RV and RA patients, wherein it was more significant in RV patients ($P \leq 0.001$). This means that IL-17 is highly associated with the development of vasculitic changes.

In agreement, Hoshino et al. [46] concluded that there is an association between IL-17 and vasculitis. There were no studies to prove otherwise.

Finally, IL-17 and NC changes were proved in our study to have a role in RV.
Drawbacks in the current study include the following: (a) the sample size of RV patients was small, and (b) RV patients were diagnosed on the basis of the clinical skin evaluation and laboratory studies, and no biopsy was done.

Conclusion
Elevated levels of serum IL-17 and characteristic NC changes raise their importance in the detection of preclinical RV.

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Conflicts of interest
There are no conflicts of interest.

References
1 Mayasoeoda E, Crowson CS, Turesson C. Incidence of extraarticular rheumatoid arthritis in Olmsted county Minnesota in 1995-2007 versus 1985-1994: a population based study. J Rheumatol 2011; 28:983–989.
2 Barids CM, Bridges J. Rheumatoid vasculitis: vanishing menace or target of a new treatment?. Curr Rheumatol Rep 2010; 12:414–419.
3 Assmann G, Pfreundschuh M, Yoswinkel J, Rituximab in patients with rheumatoid arthritis and vasculitis – associated cutaneous ulcers. Clin Exp Rheumatol 2010; 28:81–83.
4 Puechau X, Gottenberg JE, Berhelot JM. Rituximab therapy for systemic vasculitides associated with rheumatoid arthritis: results from the Autoimmunity and Rituximab Registry. Arthritis Care Res (Hoboken) 2012; 64:331–339.
5 Chen K, Carlson J. Clinical approach to cutaneous vasculitis. Am J Dermatol 2009; 9:71–92.
6 Carlson J, Chen K. Cutaneous vasculitis update: small vessel neutrophilic vasculitis syndrome. Am J Dermatol Pathol 2006; 28:486–506.
7 Chen K. The misdiagnosis of superficial thrombophlebitis as cutaneous polyanerilitis nodosa: features of intimal elastic lamina and the compact concentric muscular layer ass diagnostic pitfalls. Am J Dermatol Pathol 2010; 32:688–693.
8 Margo C, Crowson A. The spectrum of cutaneous lesions in rheumatoid arthritis: a clinical and pathological study of 43 patients. J Cutan Pathol 2003; 30:1–10.
9 Chang SH, Dong C. Signaling of interleukin-17 family cytokines in immunity and inflammation. Cell Signal 2011; 23:1069–1075.
10 Yao Z, Fanslow WC, Seldin MF. Herpesvirus Saimiri encodes a new cytokine, IL-17. Cell 1996; 85:497–506.
11 Albanecci C, Scarpioni C, Cavan I, Federici M, Nasori F, Girolomoni G. Interleukin-17 is produced by both Th1 and Th2 lymphocytes and modulates interferon-gamma and interleukin-4-induced activation of human keratinocytes. Invest Dermatol J 2000; 115:81–87.
12 Lenarczyk A, Helsloot J, Farmer K, Peters L, Sturgess A, Kirkham B. Antigen-induced IL-17 response in the peripheral blood mononuclear cells (PBMC) of healthy controls. Clin Exp Immunol 2000; 122:41–48.
13 Milner JD, Brenchley JM, Laurence A. Impaired Th17 cell differentiation in subjects with autosomal dominant hyper-IgE syndrome. Nature 2008; 452:773–776.
14 Turesson C, Jacobsson LT. Epidemiology of extra-articular manifestations in rheumatoid arthritis. Scand J Rheumatol 2004; 33:65–72.
15 Genta MS, Genta RM, Gabay C. Systemic rheumatoid vasculitis: a review. Semin Arthritis Rheum 2006; 36:88–98.
16 Hymowitz SG, Filavaroff EH, Yin JP, Lee J, Cai L, Risser P, et al. IL-17 adopt a cystine knot fold: structure and activity of a novel cytokine, IL-17 F and implication for receptor binding. EMBO J 2001; 20:5332–5341.
17 Wang X, Zhang Y, Yang XO. Infection of IL-17 and IL-17 F is controlled by conserved noncoding sequence 2. Immunity 2011; 34:149–162.
18 Wakura Y, Ishigame H, Sajo S, Naka S. Functional specialization of IL-17 family members. Immunity 2011; 34:149–162.
19 Chabaud M, Fossiez F, Taupin JL, Moussie P. Enhancing effect of IL-17 on IL-1 induced IL-6 and leukemia inhibitory factor production by rheumatoid arthritis synovioocytes and its regulation by Th2 cytokines. J Immunol 1998; 161:409–414.