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# Correlation of Serum Levels of IL-33, IL-37, Soluble Form of Vascular Endothelial Growth Factor Receptor 2 (VEGFR2), and Circulatory Frequency of VEGFR2-expressing Cells with Multiple Sclerosis Severity

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## ABSTRACT

IL-33 and IL-37 (new cytokines of IL-1 family), soluble form of vascular endothelial growth factor receptor-2 (sVEGFR2) as well as membranous expression of VEGFR2 have some key roles in the pathogenesis of autoimmune and inflammatory diseases. The aim of this study was to correlate circulatory changes of these factors with the severity of multiple sclerosis (MS) as an autoimmune and inflammatory disease.

Our case-control study was performed on 84 patients with MS and 75 healthy subjects. The serum levels of IL-33, IL-37 and sVEGFR2 in the peripheral blood samples of all participants were measured by enzyme-linked immune sorbent assay (ELISA). Flow cytometry was used to analyze the circulatory number of VEGFR2-expressing cells. The severity of MS was evaluated using the expanded disability status scale (EDSS). Finally, we evaluated the correlation between serum levels of those factors with disease severity.

Our findings showed that the serum level of IL-33, IL-37, sVEGFR2 and the circulatory number of VEGFR2-expressing cells were increased in patients with MS compared to healthy subjects ($p<0.0001$). Also, there was a significant correlation between serum levels of these 3 factors with disease severity according to EDSS.

Our study showed that the serum levels of IL-33, IL-37 and sVEGFR2 may be important prognostic biomarkers of MS.

**Keywords:** Interleukin-33, Interleukin-37; Multiple sclerosis; Soluble vascular endothelial growth factor 2; Vascular endothelial growth factor 2-expressing cells

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## INTRODUCTION

Multiple sclerosis (MS) is an inflammatory and autoimmune disease of the central nervous system (CNS)[1] causing serious motor-sensory complications.[2] The classification of MS, based on the severity of clinical symptoms, includes: relapsing remitting MS (RRMS), secondary progressive MS (SPMS), primary progressive MS (PPMS) and progressive relapsing MS (PRMS).[1] The prevalence of the disease has increased in Iran;[3,4] therefore, clarifying its underlying mechanisms seems to be necessary. Different factors such as interleukin (IL)s play an important role in the pathogens of MS; hence, in this regard, the significant increased serum level of IL-12,[5] IL-18[6], and TNF-α[7] has been reported.

IL-33, as an inflammatory member of IL-1family,[8] is released from the nucleus of cells undergoing necrosis[9] and acts as heterodimer receptor on cells expressing growth stimulation expressed gene 2 (ST2), , such as eosinophils, Th2 cells and mast cells, and plays an important role in Th2-driven diseases like asthma, allergy, and atopic dermatitis.[10] Moreover, a direct link between IL-33 and MS[11] as well as other autoimmune diseases such as rheumatoid arthritis and psoriasis[12,13] has been shown. Christophi et al showed an increased level of IL-33 in CNS as well as peripheral leukocytes of MS patients.[14] Paradoxically, another study demonstrated no increase in the level of IL-33 among MS patients compared to healthy individuals.[15]

IL-37, the other anti-inflammatory cytokine of IL-1 family,[16] is released from dendritic cells as well as peripheral blood mononuclear cells[17] during pathologies which decrease membrane integrity and thus lead to cell death. Increasing IL-4, IL-13, and IL-37 also upturn Th2 immune responses, which may play some roles in the development of Th-driven inflammatory states including Graves' disease,[18] allergic rhinitis of children,[19] acute coronary syndrome,[20] and MS.[21]

Angiogenesis, which can help to expedite the recovery of some pathologies such as brain and cardiac ischemia,[22] can also cause more tissue destruction by transferring of inflammatory cells in different pathologies such as rheumatoid arthritis,[23] diabetic retinopathy and psoriasis[24] through newly formed vessels.[25] Biologic effects of VEGF is applied at least through 2 tyrosine kinase receptors including VEGFR-1 (Flt-1) and VEGFR2 (KDR)[26] from which the main functional receptor is VEGFR2 mainly exposed on endothelium and fulfilling almost all known VEGF-mediated cellular effects.[27] A plasma soluble measurable form of VEFGR-2 (sVEGFR2) can block the binding of VEGF to its membranous receptors as a decoy[28] which may render a better clinical outcome. In fact, due to the significance of VEGFR2 on angiogenesis of tumors, there is a great interest to the monitoring of the serum levels of sVEGFR2 in cancerous patients because of its potential as a substitution bio-marker identifying the effectiveness of anti-angiogenic drugs specifically those targeting VEGF and VEGFR2.[29] Our newly published work showed that circulatory membranous as well as soluble expression of VEGFR1 increases during angiogenic and inflammatory phenomena of MS.[30] Such increase may exacerbate the symptoms and cause more disability.

Considering the effects of such cytokines and angiogenic factors in MS, we aimed to evaluate correlation of serum level of IL-33, IL-37, sVEGFR2, and circulatory number of VEGFR2-expressing cells to MS severity. Such evaluation may suggest their prognostic value and also may render some therapeutic clues.

## MATERIALS AND METHODS

### Study Subjects

This case-control study was performed on 84 patients with MS referred to the neurology clinic of the Shahid Beheshti hospital (Kashan, Iran) from January 2014 to June 2015. MS was defined according to the criteria of McDonald's. The severity of MS was evaluated using the expanded disability status scale (EDSS), a routine method for assessment of disability/severity of MS. Patients in remission phase were enrolled in the study. Any other chronic inflammatory/autoimmune diseases were considered as exclusion criteria. In addition, 75 healthy volunteers without a family history of MS and other autoimmune/chronic inflammatory diseases were considered as healthy subjects. Our study was approved by the Ethics Committee of Kashan University of Medical Sciences (No. 93219). Written informed consent from all individuals was obtained.

### Blood Samples

Blood samples were collected from all participants and serum levels of IL-37, IL-33, and VEGFR2 were measured using a commercially available enzyme-linked immune sorbent assay (ELISA) kit (My Biosource,

USA). Flow cytometry was used to enumerate VEGFR2-expressing cells. The peripheral blood mononuclear cells (PBMCs) were separated from 2 mL of anticoagulated blood using Ficoll-Hypaque (Lymphodex, Inno-Train, Germany) density gradient centrifugation. VEGFR2-expressing cells were detected by staining with an anti-human surface VEGFR2-PE antibody according to the manufacturer's instructions (R&D systems, USA). All lymphocytes in each sample were gated on the basis of light scattering properties and then at least 20,000 events were obtained to count target cells. The percentages of such cells were analyzed by a 3-color flow-cytometry using BD FACSclibur Flow Cytometer and CELL Quest software version 3 (BD, USA).

**Statistical Analysis**

The statistical indices of the serum level of IL-33,IL-37, sVEGFR2, and the circulatory number of VEGFR2 expressing-cells were analyzed by independent t and chi-square tests. Using Pearson's correlation coefficient, the correlations between variables were calculated, and simultaneous effects of various factors on these cytokines were analyzed using multiple linear regressions. Adjusted R Squared was evaluated as a criterion of goodness-of-fit test. The entire significant test was performed by alpha level set to 0.05. SPSS 16.0 (Chicago, USA) and STATA software 11, (Stata Corp., College Station, TX, USA), were used to analyze the data. Data were expressed as mean ± standard deviation (SD).

## RESULTS

**Demographics and Serum Levels of Evaluated Factors**

Basic and clinical characteristics of the patients with MS and healthy subjects are presented in Table 1. The serum levels of IL-33, IL-37, sVEGF-2, and the circulatory number of VEGFR2 expressing cells in MS patients were significantly higher than those in healthy subjects ($p \leq 0.006$).

**Table 1. Basic and clinical characteristic of multiple sclerosis (MS) patients and healthy subjects**

| | | MS patients | Healthy subjects | *p*. value |
|---|---|---|---|---|
| Number of subjects | | 84 | 75 | |
| Male/female | | 15/69 | 29/46 | 0.003 |
| Age (years)(Mean±SD) | | 34.35±9.15 | 31.8±11.8 | 0.13 |
| Family history | Positive | 7 (8.3%) | | |
| | Negative | 77 (91.7%) | | |
| Disease duration (years) | | 5.83±4.4 | | |
| Treatment duration (years) | | 3.15±3.32 | | |
| Number of recurrences | | 3.77±4.08 | | |
| Number of patients in different types of MS (%) | CIS | 5 (6) | | |
| | RRMS | 67 (79.8) | | |
| | PPMS | 2 (2.4 ) | | |
| | SPMS | 7 (8.3) | | |
| | PRMS | 3 (3.6) | | |
| Number of patients using different kinds of drugs (%) | Cinovex | 64 (76.2) | | |
| | Rebief | 10 (11.9) | | |
| | Betaferon | 2 (2.4) | | |
| | Others | 3 (3.6) | | |
| | No drug | 5 (6.1) | | |
| EDSS | CIS | 1.11 ± 0.50 | | |
| | RRMS | 2.07 ± 1.447 | | |
| | PPMS | 6.00 ± 0.00 | | |
| | SPMS | 4.93 ± 1.427 | | |
| | PRMS | 6.00 ± 0.50 | | |
| IL-33 | | 62.27 ± 26.47 | 19.36±7.64 | $p<0.0001$ |
| IL-37 | | 95.7 ± 36.13 | 36.6±15.23 | $p<0.0001$ |
| sVEGFR2 | | 10.53 ± 10.66 | 7.20 ± 2.17 | $p= 0.006$ |
| VEGFR2-expressing cells frequency | | 3.59 ± 1.57 | 1.76 ± .93 | $p<0.001$ |

CIS: clinical isolated syndrome, RRMS: relapsing remitting multiple sclerosis, PPMS: primary progressive multiple sclerosis, SPMS: secondary progressive multiple sclerosis, sVEGFR2: soluble vascular endothelial growth factor 2, EDSS: expanded disability status scale

**Association of Serum Levels of IL-33, IL-37, sVEGF-2, and the Number of VEGFR2expressing Cells with Basic and Clinical Characteristic of MS Patients**

The serum levels of IL-33, IL-37, sVEGF-2, and the circulatory number of VEGFR2-expressing cells were evaluated according to EDSS, type of MS, gender, family history, disease duration, treatment duration, number of recurrences, and kind of medication. The serum levels of IL-33 were significantly higher in severe forms of MS (EDSS=5-9.5) than those in mild forms (EDSS=0-4.5) ($p \leq 0.001$) according to EDSS score (Table 2).

**Table 2. Values of IL-33, IL-37, sVEGFR2, and the frequency of VEGFR2-expressing cells according to different variables in MS patients**

| | | IL-33 | *p*. value | IL-37 | *p*. value | sVEGFR2 | *p*. value | VEGFR2-expressing cells | *p*. value |
|---|---|---|---|---|---|---|---|---|---|
| EDSS | Mild (0.5-4.5) | 63.60± 19.97 | <0.0001 | 105.58±31.83 | 0.874 | 11.15±13.43 | 0.905 | 3.70±1.33 | 0.061 |
| | Sever (5- 9.5) | 88.22±26.01 | | 107.06±33.73 | | 10.75±2.01 | | 4.65±1.77 | |
| Type | CIS | 31.41±21.62 | <0.0001 | 72.59± 38.31 | 0.112 | 8.72±1.63 | 0.022 | 3.80±1.31 | 0.540 |
| | RRMS | 59.00±22.16 | | 93.77 ±35.48 | | 10.64±11.90 | | 3.48±1.56 | |
| | PPMS | 65.54 ±7.65 | | 140.48±46.68 | | 7.40±0.70 | | 5.35±1.34 | |
| | SPMS | 105.99 ±20.69 | | 114.84±32.81 | | 10.95±1.99 | | 4.10±1.98 | |
| | PRMS | 82.62 ±23.71 | | 102.83±23.74 | | 12.30±0.60 | | 3.33±0.56 | |
| Sex | Male | 62.18±27.14 | 0.944 | 95.77±36.96 | 0.971 | 9.43±1.93 | 0.661 | 3.25±1.21 | 0.359 |
| | Female | 62.71±23.97 | | 95.40±33.2 | | 10.77±11.73 | | 3.67±1.63 | |
| Family history | No | 62.15±27.12 | 0.891 | 95.85±36.15 | 0.899 | 10.57±11.12 | 0.919 | 3.51±1.57 | 0.117 |
| | Yes | 63.6±19.4 | | 94.02±38.7 | | 10.14±2.37 | | 4.48±1.35 | |
| Disease duration (years) | Control group | 19.36 ±7.64 | <0.0001 | 36.63±15.23 | <0.0001 | 7.20 ±2.17 | 0.007 | 1.76 ±0.93 | 0.0001 |
| | 1-5 | 48.39 ±18.90 | | 86.52 ±34.66 | | 9.08 ±2.24 | | 3.37 ±1.45 | |
| | 6-10 | 78.00 ±26.10 | | 103.27±34.94 | | 13.15±18.84 | | 4.07 ±1.75 | |
| | 11< | 84.39 ±19.55 | | 117.04±34.79 | | 10.55±2.15 | | 3.40±1.46 | |
| Treatment duration (years) | 0-6 | 42.36±12.34 | 0.124 | 71.89 ±17.02 | 0.159 | 9.46±1.95 | 0.861 | 3.11±1.24 | 0.404 |
| | 7-12 | 59.53±22.46 | | 92.84±29.97 | | 9.68±2.38 | | 3.87±0.78 | |
| | 13-17 | 80.53±23.16 | | 108.28±27.78 | | 9,84±2,83 | | 4,32± 0.4 | |
| Number of recurrences | 0-1 | 46.86 ±18.32 | <0.0001 | 82.15 ±36.31 | 0.015 | 9.08± 1.98 | 0.059 | 3.48 ±1.33 | 0.044 |
| | 2-5 | 61.22 ±24.37 | | 96.31± 33.08 | | 9.11±2.37 | | 3.30±1.46 | |
| | 6< | 86.87±23.63 | | 113.91±35.95 | | 15.81±22.33 | | 4.39±1.89 | |
| Drugs | No drug | 75.04±32.72 | 0.712 | 93.22 ±33.12 | 0.338 | 9.74±1.27 | 0.971 | 3.80±1.79 | 0.128 |
| | Cinovex | 60.89 ±26.41 | | 92.61±35.48 | | 10.85±12.15 | | 3.39±1.56 | |
| | Rebief | 64.35±28.06 | | 104.32±35.93 | | 9.38 ±2.71 | | 4.15±1.31 | |
| | Others | 63.08±21.55 | | 120.46±46.34 | | 9.56±2.52 | | 4.86±1.39 | |

CIS: clinical isolated syndrome, RRMS: relapsing remitting multiple sclerosis, PPMS: primary progressive multiple sclerosis, SPMS: secondary progressive multiple sclerosis, PRMS: progressive relapsing multiple sclerosis, EDSS: expanded disability status scale, sVEGFR2: soluble vascular endothelial growth factor 2

**Correlation of Serum Levels of IL-33, IL-37, sVEGFR2, and the Number of VEGFR2-expressing Cells with Different Parameters**

There were significant correlations between the serum levels of IL-33, IL-37, and sVEGFR2 with disease severity according to EDSS score. Every-unit increase in the serum level of sVEGFR2 raised the disability at nearly 0.28 unit according to EDSS score in univariate model ($p$=0.001); this coefficient changed to 0.125 unit in multivariable model (Table 3).

Linear multiple regression analysis showed that the factors of age, familial history, number of relapses, serum levels of IL-33, IL-37, and sVEGFR2 have a positive effect on EDSS (Table 4). Such model showed a high goodness of fit (Adjusted $R^2$=0.922). Modeling the effects of serum levels of IL-33, IL-37 and sVEGFR2 on MS severity, we found a high goodness of fit (Adjusted $R^2$=0.82). We also demonstrated that the combination of serum levels of IL-33 and IL-37 shows a proper criterion in the evaluation of MS severity (Adjusted $R^2$=0.819). In the latter model, there was multicollinearity between IL-33 and IL-37. Therefore, omitting IL-37, we established a model using only IL-33 assessing MS severity according to EDSS (Adjusted $R^2$=0.821).

**Table 3. Correlation between the serum levels of IL-33, IL-37, sVEGFR2, and the number of VEGFR2-expressing cells with different parameters in multiple sclerosis (MS) patients**

| | IL-33 | | IL-37 | | sVEGFR2 | | Number of VEGFR2-expressing cells | |
|---|---|---|---|---|---|---|---|---|
| | Correlation coefficient | *p*. value | Correlation coefficient | *p*. value | Correlation coefficient | *p*. value | Correlation coefficient | *p*. value |
| Age | 0.238 | 0.003 | 0.175 | 0.028 | 0.189 | 0.017 | 0.019 | 0.818 |
| EDSS | 0.736 | 0.0001 | 0.553 | 0.000 | 0.498 | 0.000 | 0.115 | 0.297 |
| Number of relapses | 0.388 | 0.0001 | 0.180 | 0.101 | 0.289 | 0.008 | 0.151 | 0.169 |
| Treatment duration | 0.4 | 0.0001 | 0.308 | 0.005 | 0.106 | 0.339 | 0.190 | 0.085 |
| Disease duration | 0.501 | 0.0001 | 0.293 | 0.007 | 0.086 | 0.437 | 0.123 | 0.263 |

EDSS: expanded disability status scale, sVEGFR2: soluble vascular endothelial growth factor 2

**Table 4. Linear multiple regression analysis evaluating the effect of EDSS on the IL-33, IL-37, and sVEGFR2 in MS patients**

| | Unstandardized Coefficients | | Standardized Coefficients | t | Sig. |
|---|---|---|---|---|---|
| variables | B | Std. Error | Beta | | |
| Age | 0.038 | 0.01 | 0.44 | 3.886 | 0 |
| Family history | 1.099 | 0.379 | 0.096 | 2.897 | 0.005 |
| CIS | -2.796 | 0.482 | -0.223 | -5.806 | 0 |
| RRMS | -2.022 | 0.227 | -0.582 | -8.89 | 0 |
| Relapse number | 0.068 | 0.027 | 0.122 | 2.562 | 0.012 |
| IL-33 | 0.012 | 0.006 | 0.259 | 2.15 | 0.035 |
| IL-37 | 0.012 | 0.003 | 0.389 | 3.511 | 0.001 |
| sVEGFR2 | 0.205 | 0.073 | 0.258 | 2.806 | 0.006 |

EDSS: expanded disability status scale, RRMS: relapsing remitting multiple sclerosis, sVEGFR2: soluble vascular endothelial growth factor 2, CIS: clinical isolated syndrome, sig: significance, Std. Error: standard error

## DISCUSSION

Serum level of IL-33 has a direct relationship to the severity of some inflammatory/autoimmune diseases such as rheumatoid arthritis,[31] ankylosing spondylitis,[32] Behcet's disease,[33] primary Sjögren syndrome,[34] cardiovascular disorders in patients with rheumatoid arthritis,[35] inflammatory bowel disease,[36] asthma[37], and MS.[11] Zarpelon et al. evaluated IL-33/ST2 signaling in an animal model of inflammation pain and observed that this axis has an important role in induction of inflammation.[38] In line with such studies, we showed an increased serum level of IL-33 in MS patients correlated to disease severity. Such association has also been shown in the case of other inflammatory cytokines such as TNF-α[39] and IL-6.[40]

Confirming the results of a recent study,[21] our data showed that serum level of IL-37 was elevated in patients with MS correlated to disease severity. Anti-inflammatory properties of IL-10[41] and IL-37[42] may support their elevation in inflammatory condition of MS to reimburse the magnitude of inflammation. Such elevation has also been shown in other inflammatory conditions such as ankylosing spondylitis to inhibit the inflammatory cytokines such as TNF-α,[43] rheumatoid arthritis,[44] and asthma.[45] The inhibitory effect of IL-37 could be imposed by inhibition of both NF-κB signaling pathway[46] and IL-1R8 and IL-18Rα receptors[47] as protective mechanisms which reduce the pathogenesis of the disease. The above-mentioned concept may confirm the beneficial effect of IL-37 as a novel therapy in autoimmune diseases like MS.

We showed a significant increase in serum level of sVEGFR2 correlated to the severity of the patients according to EDSS score as well as the type of MS, i.e. it was significantly lower in mild forms of MS than that in severe forms. Considering sVEGFR2 as a decoy preventing the binding of VEGF to VEGFR2, one may consider such correlation as a protective mechanism, yet insufficient to relieve the disease progression. In this regard, sVEGFR2 may be produced to modulate angiogenic effects or, inflammatory effects of VEGF or both .[48] It means that in more severe types of MS, more *de novo* or induced production of sVEGFR2 constitutes a regulatory mechanism which tries to improve the disease through prevention of both MS progression and lesion formation.[49] Moreover, this prognostic approach may support the idea that a more aggressive therapeutic regimen should be started in patients with highly elevated serum sVEGFR2 levels early in their disease course. Such prognostic concept in the clinical outcome of MS patients has also been indicated in the case of tumors.[28] Moreover; sVEGFR2 may replace EDSS, as it is more objective rather than subjective.

We may also consider the correlation between sVEGFR2 and clinical outcome of the MS as estimation for the extent of the inhibitory effects of factors blocking the binding of VEGFR2 to VEGF such as bevacizumab and also blocker agents of tyrosine kinase activity involved in intracellular signaling of VEGFR2 such as sunitinib and sorafenib. Such estimation helps us to evaluate introducing those factors as possible new therapeutics for MS. It should be noted that, recently, inhibition of angiogenesis generally, and VEGFR2 specifically, are considered as therapeutic targets in the treatment of some autoimmunities such as rheumatoid arthritis as well as cancers.[50-52] Interestingly, selective inhibition of VEGFR2 present on macrophages also has therapeutic potential values.[35]

Finding a strong correlation between each of IL-33, IL-37, sVEGFR2, and the circulatory number of VEGFR2-expressing cells with EDSS after adjusting the effects of confounding factors, we showed that IL-33 may be a strong predictor of severe forms of MS independent of sex, age, number of recurrences, and disease duration. This finding would be of more importance considering the fact that such combination does not necessitate evaluating the number of VEGFR2 expressing cells, whose measurement is expensive and is not available in most routine laboratories. Other factors except the evaluated ones in our study may probably affect the MS severity which should be defined in other studies.

The limitation of our study was lack of monitoring serum levels of IL-33, IL-37, sVEGFR2, and the circulatory number of VEGFR2-expressing cells longitudinally. This limitation allowed just a cross-sectional analysis of their changes of only limited robustness. Secondly, functional assays which provide further information on their possible immunoregulatory mechanisms were not performed.

Our finding shows that elevation of serum level of IL-33, IL-37, and sVEGFR2 in patients with MS is correlated to disease severity. These findings may be used for both diagnostic approaches of MS severity and

therapeutic strategies.

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## REFERENCES

1. Goldenberg MM. Multiple sclerosis review. P T 2012; 37(3):175-84.
2. Murray TJ. Diagnosis and treatment of multiple sclerosis. BMJ 2006; 332(7540):525-7.
3. Etemadifar M, Maghzi A-H. Sharp increase in the incidence and prevalence of multiple sclerosis in Isfahan, Iran. Mult Scler 2011; 17(8):1022-7.
4. Moghtaderi A, Rakhshanizadeh F, Shahraki-Ibrahimi S. Incidence and prevalence of multiple sclerosis in southeastern Iran. Clin Neurol Neurosurg 2013; 115.
5. Balashov KE, Smith DR, Khoury SJ, Hafler DA, Weiner HL. Increased interleukin 12 production in progressive multiple sclerosis: induction by activated CD4+ T cells via CD40 ligand. J Proc Natl Acad Sci U S A 1997; 94(2):599-603.
6. Nicoletti F, Di Marco R, Mangano K, Patti F, Reggio E, Nicoletti A, et al. Increased serum levels of interleukin-18 in patients with multiple sclerosis. Neurology 2001; 57(2):342-4.
7. Mikova O, Yakimova R, Bosmans E, Kenis G, Maes M. Increased serum tumor necrosis factor alpha concentrations in major depression and multiple sclerosis. Eur Neuropsychopharmacol 2001; 11(3):203-8.
8. Schmitz J, Owyang A, Oldham E, Song Y, Murphy E, McClanahan TK, et al. IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. Immunity 2005; 23(5):479-90.
9. Kakkar R, Hei H, Dobner S, Lee RT. Interleukin 33 as a mechanically responsive cytokine secreted by living cells. J Biol Chem 2012; 287(9):6941–8.
10. Mirchandani AS, Salmond RJ, Liew FY. Interleukin-33 and the function of innate lymphoid cells. Trends Immunol 2012; 33(8):389–96
11. Christophi GP, Gruber RC, Panos M, Christophi RL, Jubelt B, Massa PT. Interleukin-33 upregulation in peripheral leukocytes and CNS of multiple sclerosis patients. Clin Immunol 2012; 142(3):308-19.
12. Suttle MM, Nilsson G, Snellman E, Harvima I. Experimentally induced psoriatic lesion associates with interleukin (IL)-6 in mast cells and appearance of dermal cells expressing IL-33 and IL-6 receptor. Clin Exp Immunol 2012; 169(3):311-9.
13. Talabot-Ayer D, McKee T, Gindre P, Bas S, Baeten DL, Gabay C, et al. Distinct serum and synovial fluid interleukin (IL)-33 levels in rheumatoid arthritis, psoriatic arthritis and osteoarthritis. J Joint Bone Spine 2012; 79(1):32-7.
14. Christophi GP, Gruber RC, Panos M, Christophi RL, Jubelt B, Massa PT. Interleukin-33 upregulation in peripheral leukocytes and CNS of multiple sclerosis patients. Clin Immunol 2012; 142(3):308-19.
15. Zhang F, Tossberg JT, Spurlock CF, Yao SY, Aune TM, Sriram S. Expression of IL-33 and its epigenetic regulation in multiple sclerosis. Ann Clin Transl Neurol 2014; 1(5):307-18.
16. Sharma S, Kulk N, Nold MF, Gräf R, Kim S-H, Reinhardt D, et al. The IL-1 family member 7b translocates to the nucleus and down-regulates proinflammatory cytokines. J Immunol 2008; 180(8):5477-82.
17. Bufler P, Gamboni-Robertson F, Azam T, Kim SH, Dinarello CA. Interleukin-1 homologues IL-1F7b and IL-18 contain functional mRNA instability elements within the coding region. responsive to lipopolysaccharide. Biochem J 2004; 381(pt2):503-10.
18. Li Y, Wang Z, Yu T, Chen B, Zhang J, Huang K, et al. Increased expression of IL-37 in patients with Graves' disease and its contribution to suppression of proinflammatory cytokines production in peripheral blood mononuclear cells. PLoS One 2014; 9(9):e107183.
19. Liu W, Deng L, Chen Y, Sun C, Wang J, Zhou L, et al. Anti-Inflammatory Effect of IL-37b in Children with Allergic Rhinitis. Mediators Inflamm 2014; 2014:746846.
20. Ji Q, Zeng Q, Huang Y, Shi Y, Lin Y, Lu Z, et al. Elevated plasma IL-37, IL-18, and IL-18BP concentrations in patients with acute coronary syndrome. Mediators Inflamm 2014; 2014, 165742.
21. Farrokhi M, Rezaei A, Amani-Beni A, Etemadifar M, Kouchaki E, Zahedi A. Increased serum level of IL-37 in patients with multiple sclerosis and neuromyelitis optica. Acta Neurol Belg 2015; 115(4):609-14.
22. Plate KH. Mechanisms of angiogenesis in the brain. J Neuropathol Exp Neurol 1999; 58(4): 313-20.
23. Folkman J, Shing Y. Angiogenesis. J Biol Chem 1992; 267(16):10931-4.
24. Creamer D, Sullivan D, Bicknell R, Barker J. J Angiogenesis in psoriasis. Angiogenesis 2002; 5(4):231-6.
25. Kirk SL, Karlik SJ. VEGF and vascular changes in chronic neuroinflammation. J Autoimmun 2003; 21(4):353-63.

26. Robinson CJ, Stringer SE. The splice variants of vascular endothelial growth factor (VEGF) and their receptors. J Cell Sci 2001; 114(Pt 5):853-65.
27. Kasap M, Sazci A. The comparison of VEGFR-1-binding domain of VEGF-A with modelled VEGF-C sheds light on receptor specificity. J Theor Biol 2008; 253(3):446-51.
28. Ebos JM, Bocci G, Man S, Thorpe PE, Hicklin DJ, Zhou D, Jia X, Kerbel RS. A naturally occurring soluble form of vascular endothelial growth factor receptor 2 detected in mouse and human plasma. Mol Cancer Res 2004; 2(6):315-26.
29. Ebos JM, Lee CR, Bogdanovic E, Alami J, Van Slyke P, Francia G, et al. Vascular endothelial growth factor-mediated decrease in plasma soluble vascular endothelial growth factor receptor-2 levels as a surrogate biomarker for tumor growth. Cancer Res 2008; 68(2):521-9.
30. Kouchaki E, Otroshi Shahreza B, Faraji S, Nikoueinejad H, Sehat M. Iran J Allergy Asthma Immunol 2016; 15(3):204-11.
31. Matsuyama Y, Okazaki H, Tamemoto H, Kimura H, Kamata Y, Nagatani K, et al. Increased levels of interleukin 33 in sera and synovial fluid from patients with active rheumatoid arthritis. J Rheumatol 2010; 37(1):18-25.
32. Han GW, Zeng LW, Liang CX, Cheng BL, Yu BS, Li HM, et al. Serum levels of IL-33 is increased in patients with ankylosing spondylitis. Clin Rheumatol 2011; 30(12):1583-8.
33. Hamzaoui K, Kaabachi W, Fazaa B, Zakraoui L, Mili-Boussen I, Haj Sassi F. Serum IL-33 levels and skin mRNA expression in Behcet's disease. Clin Exp Rheumatol 2012; 31(3 Suppl 77):6-14.
34. Jung SM, Lee J, Baek SY, Lee JH, Lee J, Park KS, et al. The Interleukin 33/ST2 Axis in Patients with Primary Sjögren Syndrome: Expression in Serum and Salivary Glands, and the Clinical Association. J Rheumatol 2015; 42(2):264-71.
35. Shen J, Shang Q, Wong CK, Li EK, Wang S, Li RJ, et al. IL-33 and soluble ST2 levels as novel predictors for remission and progression of carotid plaque in early rheumatoid arthritis: A prospective study. Semin Arthritis Rheum 2015; 45(1):18-27.
36. Beltrán CJ, Núñez LE, Díaz-Jiménez D, Farfan N, Candia E, Heine C, et al. Characterization of the novel ST2/IL-33 system in patients with inflammatory bowel disease. Inflamm Bowel Dis 2010; 16(7):1097-107.
37. Kearley J, Buckland KF, Mathie SA, Lloyd CM. Resolution of Allergic Inflammation and Airway Hyperreactivity Is Dependent upon Disruption of the T1/ST2–IL-33 Pathway. Am J Respir Crit Care Med 2009; 179(9):772-81.
38. Zarpelon A, Cunha T, Alves-Filho J, Pinto L, Ferreira S, McInnes I, et al. IL-33/ST2 signalling contributes to carrageenin-induced innate inflammation and inflammatory pain: role of cytokines, endothelin-1 and prostaglandin E2. Br J Pharmacol 2013; 169(1):90-101.
39. Sharief MK, Hentges R. Association between tumor necrosis factor-α and disease progression in patients with multiple sclerosis. N Engl J Med 1991; 325(7):467-72.
40. Padberg F, Feneberg W, Schmidt S, Schwarz M, Körschenhausen D, Greenberg B, et al. CSF and serum levels of soluble interleukin-6 receptors (sIL-6R and sgp130), but not of interleukin-6 are altered in multiple sclerosis. J neuroimmunol 1999; 99(2):218-23.
41. Luomala M, Lehtimäki T, Huhtala H, Ukkonen M, Koivula T, Hurme M, et al. Promoter polymorphism of IL-10 and severity of multiple sclerosis. Acta Neurol Scand 2003; 108(6):396-400.
42. Nold MF, Nold-Petry CA, Zepp JA, Palmer BE, Bufler P, Dinarello CA. IL-37 is a fundamental inhibitor of innate immunity. Nat Immunol 2010;11(11):1014-22.
43. Chen B, Huang K, Ye L, Li Y, Zhang J, Zhang J, et al. Interleukin-37 is increased in ankylosing spondylitis patients and associated with disease activity. J Transl Med 2015; 13(1):1-9.
44. Xia L, Shen H, Lu J. Elevated serum and synovial fluid levels of interleukin-37 in patients with rheumatoid arthritis: Attenuated the production of inflammatory cytokines. Cytokine 2015; 76(2):553-7.
45. Charrad R, Berraïes A, Hamdi B, Ammar J, Hamzaoui K, Hamzaoui A. Anti-Inflammatory activity of IL-37 in Asthmatic Children: Correlation with Inflammatory Cytokines TNF-α, IL-β, IL-6 and IL-17A. Immunobiology 2015; 221(2):182-7.
46. Xu D, Wang A, Jiang F, Hu J, Zhang X. Effects of interleukin-37 on cardiac function after myocardial infarction in mice. Int J Clin Exp Pathol 2015; 8(5):5247-51.
47. Nold-Petry CA, Lo CY, Rudloff I, Elgass KD, Li S, Gantier MP, et al. IL-37 requires the receptors IL-18R [alpha] and IL-1R8 (SIGIRR) to carry out its multifaceted anti-inflammatory program upon innate signal transduction. Nat Immunol 2015; 16(4):354-65.
48. Ballara S, Taylor PC, Reusch P, Marme D, Feldmann M, Maini RN, Paleolog EM. Raised serum vascular endothelial growth factor levels are associated with destructive change in inflammatory arthritis. Arthritis Rheum 2001; 44(9):2055-64.
49. Miotla J, Maciewicz R, Kendrew J, Feldmann M,

Paleolog E. Treatment with soluble VEGF receptor reduces disease severity in murine collagen-induced arthritis. Lab Invest 2000; 80(8):1195-205.

50. Ahnert P, Kirsten H. Association of ITGAV supports a role of angiogenesis in rheumatoid arthritis. Arthritis Res Ther 2007; 9(5):108.
51. Koch AE, Distler O. Vasculopathy and disordered angiogenesis in selected rheumatic diseases: rheumatoid arthritis and systemic sclerosis. Arthritis Res Ther 2007; 9 Suppl 2:S3.
52. Holmes K, Roberts OL, Thomas AM, Cross MJ. Vascular endothelial growth factor receptor-2: structure, function, intracellular signalling and therapeutic inhibition. Cell Signal 2007; 19(10):2003-12.