Journal of Arthritis
ISSN: 2167-7921
Arthritis

Saber et al., J Arthritis 2015, 4:3
http://dx.doi.org/10.4172/2167-7921.1000167

Research Article Open Access

Joint Destruction and Osteoporosis are Associated with Upregulation of IL-34 and Cathepsin k Expression in Rheumatoid Arthritis. Clinical Trial with Anti TNF α Therapy

Nayera Saber[1]*, Mary Atef[2] and Doaa Abdel Aziz[3]

[1]*Assistant professor of Rheumatology and Rehabilitation, Faculty of Medicine, Ain Shams University, Egypt*
[2]*Lecturer of Rheumatology and Rehabilitation, Faculty of Medicine, Ain Shams University, Egypt*
[3]*Lecturer Faculty of of Clinical Pathology, Faculty of Medicine, Ain Shams University, Egypt*

Abstract

Background: Previous studies demonstrated that significant association was found between IL-34 synovial tissue expression and synovitis severity in RA. Furthermore the overexpression of cathepsin K in RA synovia proves that this protease may become a new and highly specific biomarker for RA.

Objective: To find out whether serum levels of IL-34 and Cathepsin-K vary in patients with longstanding RA treated with biologic therapy versus early RA patients treated with conventional DMARDS. Also to estimate any association between their baseline serum levels and disease activity, subsequent joint destruction and osteoporosis.

Methods: This study included forty one RA patients, 21 as a patient group who started treatment with anti TNFα therapy. Group of controls included 20 RA patients who were treated with DMARDs. Full clinical and laboratory assessment as well as radiological by Van Der Heide Sharp score (SHS) and DEXA T scores were done. Serum IL34 and cathepsin k were assessed by ELISA before and one year after treatment.

Results: After one year of therapy, patients group had significant lower serum IL-34 (s.IL-34) level, cathepsin K level CRP, DAS28, DEXA T-score, and SHS. ($p<0.01$) than baseline values($p<0.01$), while no significant change in s.IL-34, cathepsin-K in controls. Baseline sIL34 and cathepsin K were positively correlated with DAS28 and SHS and DEXA T scores ($p<0.05$). There was a significant difference in morning stiffness, DAS28, and serum IL-34 in good responders versus poor responders according to WHO/ILAR response criteria of improvement among patients group. High baseline DAS28 is independent risk factors for radiographic change in RA while high baseline CRP is a risk factor for osteoporosis in RA patients.

Conclusion: Serum IL 34 and cathepsin k were strongly linked to disease activity and duration in RA patients and were highly relevant to both localized osteoporosis and generalized osteoporosis. Also, Anti TNFα therapy effectively decrease both biomarkers regardless drug type, with amelioration of clinical, laboratory and radiological parameters of RA patients.

Keywords: Rheumatoid arthritis; Joint destruction; Osteoporosis; Il34; Cathepsin K; Anti Tnfα drugs

Introduction

Rheumatoid arthritis (RA) is an autoimmune chronic inflammatory disease characterized by inflammatory infiltration of the synovium and synovial hyperplasia, leading to cartilage degradation and bone destruction which may be manifested as erosions, localized juxta-articular bone loss, or generalized bone loss [1]. Because functional outcome in RA is dependent on the extent of joint destruction, early diagnosis and immediate aggressive treatment of RA are required to prevent progressive joint damage, functional disability and reduced quality of life [2].

Osteoclasts (OCs) differentiate from the monocyte/macrophage lineage of hematopoietic myeloid progenitors and its differentiation correlates with the severity of the inflammatory condition. Receptor activator of nuclear factor-κB ligand (RANKL) and macrophage colony-stimulating factor (M-CSF) (also called CSF-1) are considered crucial in osteoclast differentiation and function [3]. Imbalance between osteoblast and osteoclast activities due to inflammatory cytokines pannus interface, periarticular and generalized bone loss in RA pathophysiology [5]. Tumor necrosis factor alpha (TNF−α) is mediating mobilization of osteoclast precursors (OCPs) from bone marrow into the inflamed joint. Also, it stimulates fibroblast-like synovial cells (FLS) to increase cytokines production, which accelerates OC activation in the inflamed synovium of RA [6].

Interleukin−34 (IL-34) is a newly discovered cytokine [7]. It shares a common receptor (c-Fms) with M-CSF, which is expressed on the cell surface of human monocytes [8]. Although both of them share the same receptor, their signal transduction mechanisms and biological activity are not identical [9].

Studies showed that IL-34 can stimulate colony formation of macrophages in human bone marrow cells in a similar efficacy like M-CSF and can substitute entirely for it in RANKL-induced osteoclastogenesis [10]. Chemel et al., demonstrated that significant

***Corresponding author:** Nayera Saber, Assistant Professor, Ain Shams University Faculty of Medicine, Rheumatology, Egypt, Tel: +201117288825; E-mail: dr.nayera_abdelmoaty@med.asu.edu.eg

Received August 17, 2015; **Accepted** September 08, 2015; **Published** September 19, 2015

Citation: Saber N, Atef M, AbdelAziz D (2015) Joint Destruction and Osteoporosis are Associated with Upregulation of IL-34 and Cathepsin k Expression in Rheumatoid Arthritis. Clinical Trial with Anti TNF α Therapy. J Arthritis 4: 167. doi:10.4172/2167-7921.1000167

association was found between IL-34 synovial tissue expression and synovitis severity in RA [11]. Eda et al., documented that IL-34 could increase IL-6 and chemokine levels in human whole blood [12]. It also could promote osteoclastogenesis *in vitro* [13]. Tian et al., found that IL-34 expression could be induced by TNF-α and IL-1β which are pivotal cytokines in RA [14]. Studies have shown that administration of an antibody or inhibitor against the c-Fms selectively and completely blocks osteoclastogenesis and bone erosion induced by TNF–α. Thus, identifying factors involved in TNFα- induced octeoclastogenesis that contribute to erosive arthritis is of great clinical importance [15]. Furthermore, the administration of TNFα blocking agents results in a decrease in the RA inflammatory responses and provides a clinical improvement [16].

Cathepsins (B, L, S, K) are an extensive family of cysteine proteases that are regulated by cytokines and have broad proteolytic activity including activity on types II, IX, and XI collagen and proteoglycans [17]. Cathepsin K is considered to be one of the most important proteolytic enzymes in osteoclastic bone resorption as it can degrade type I collagen at multiple sites in the triple helical domains [18]. Cathepsin K enzyme is abundantly detected in osteoclasts along the bone resorption surfaces, in intracellular lysosomes and transcytotic vesicles [19].

It is expressed by both macrophages and fibroblasts in RA synovial tissue and is present in significantly higher concentrations than in osteoarthritis (OA) [20].

Cathepsins potential role as mediators of bone destruction in arthritis was confirmed in studies in which a cysteine protease inhibitor significantly decreased joint damage in the animal arthritis model [21]. Besides collagens, cathepsin K cleaves a variety of other bone- and cartilage resident proteins such as osteonectin, aggrecan, and IGF-1 [22,23].

In this context, we inquire whether IL-34 and cathepsin K ,two different inflammatory mediators have synergistic roles in RA pathogenesis and what will be the effect of administration of TNFα inhibitors drugs on localized and generalized osteoporosis.

The objective of the present study is to find out whether serum levels of IL-34 and Cathepsin-K vary in patients with longstanding RA treated with biologic therapy versus early RA patients treated with conventional DMARDS. Also to estimate any association between their baseline serum levels and disease activity, subsequent joint destruction and osteoporosis.

Methods

This study included 41 RA patients presented to outpatient clinic of Physical Medicine, Rheumatology and Rehabilitation Department of Ain Shams University Hospitals.

Inclusion criteria

RA patients fulfilled the American College of Rheumatology (ACR) and ACR/European League Against Rheumatism (EULAR) 2010 criteria for RA [24,25], age > 18 years , disease activity score (DAS28) > 3.2. They were classified into either cases or controls according to disease duration. Group of cases included 21 longstanding (> 5years) RA patients who will receive only biologic therapy (anti-TNFα) either etanercept (Enbrel) or adalimumab (Humira). Group of controls included 20 short duration (< 5years) RA patients who will receive DMARDs (monotherapy or combined). They were followed up for 1 year.

The study was conducted in accordance with the World Medical Association Declaration of Helsinki for human subjects and the study was approved by the ethics committee of the faculty of Medicine and all patients were informed and gave their written consent.

Exclusion criteria

Patients who had Paget disease, multiple myeloma, breast cancer, bone metastasis or Patients on medication that influence bone metabolism as: glucocorticoid, heparin, anticonvulsant, thyroxin, hormone replacement therapy or any drug used in treatment of osteoporosis or previously received any anti TNFα therapy were excluded from the study.

Thorough clinical assessment

All patients were subjected to the following before starting treatment and 1 year after, full medical history taking with special emphasis on: age, sex, disease duration, morning stiffness duration, global pain assessment using Visual Analog Scale (VAS, 0-10) cm, modified Health Assessment Questionnaire (MHAQ) Disability Index [26] and type of drug therapy. Tender joint count (TJC), swollen joint count (SJC) and Extra- articular manifestations (EAM).

Complete blood count (CBC) was done including haemoglobulin level. Erythrocyte sedimentation rate (ESR) by the Westergren method. C–Reactive Protein (CRP) level by nephlometry and rheumatoid factor (RF) by qualitative method. The 28-joint count Disease Activity Score (DAS28) was calculated using (CRP) level [27]. Disease activity status of remission (REM), low disease activity (LDA), moderate disease activity (MDA), and high disease activity (HDA) were also determined using DAS28. REM was defined as DAS 28 < 2.6, LDA as 2.6 ≤ DAS28 ≤ 3.2, MDA as 3.2 < DAS28 < 5.1, HDA as 5.1 ≤ DAS28 [28].

Radiological investigations

Plain X–ray: P-A view of both hands and wrists were assessed using the Van Der Heide Sharp score (SHS) for the extent of joint damage, as indicated by joint space narrowing and erosion. Sixteen joint areas and 15 joint areas in each hand were scored for erosion and joint space narrowing, respectively. The total Sharp Score (TSS) was calculated as the sum of the erosion and joint space narrowing scores [29]. ΔSHS ≥ 1 unit/year was regarded as radiographic progression according to the previously used definition [30].

Dual-energy X-ray absorptiometry (DEXA): By using GE Medical Systems, LUNAR (DPX–MD+) device. Bone mineral density (BMD) was measured before and after treatment at 3 sites which are lumbar spine, femoral neck, and distal radius. World Health Organization (WHO) diagnosis T score criteria were applied to BMD measurement [31].

Measurement of serum IL–34 and Cathepsin k levels: Serum samples were obtained from 30 patients with RA before starting treatment and 1 year after. Serum samples were stored at –80°C until analysis.Interleukin-34 (IL-34) and Cathepsin K(Cath-K) have been estimated by using sandwich enzyme immunoassay (ELISA) technique (WKEAMedSupplies, Changchun,China) as supplied with kit from WKEAMedSupplies Company, China (tom.wkea@hotmail.com). All biochemical measures were performed in a single batch and a comparable number of patient and control samples were always assayed simultaneously in the same ELISA plate. Cut off points in ng/L were calculated from our control group.

Statistical analysis

IBM SPSS statistics (V. 22.0, IBM Corp., USA, 2013) was used

for data analysis. Data were expressed as Mean ± SD for quantitative parametric measures in addition to Median and Percentiles for quantitative non-parametric measures.

The following tests were done:

1. Comparison between two independent groups for non-parametric data using Wilcoxon Rank Sum test.
2. Comparison between more than 2 patient groups for non-parametric data using Kruskall Wallis test.
3. Ranked Spearman correlation test to study the possible association between each two variables among each group for non-parametric data.
4. Diagnostic sensitivity, specificity, predictive values, efficacy and accuracy by diagnostic validity test.
5. Logistic Multi-Regression analysis was used to search for a panel (independent parameters) that can predict the dependent variable parameter. The probability of error at 0.05 was considered sig., while at 0.01 and 0.001 are highly significant.

Results

The study included 41 RA patients enrolled from Physical Medicine, Rheumatology and Rehabilitation Department, Ain Shams University. They were randomly classified according to type of treatment into: group of patients included 21 RA patients: 17 females (80.95%) and 3 males (14.28%) started treatment with anti TNFα therapy either etanercept (Enbrel) or adalimumab (Humira). Group of controls included 20 RA patients, 14 females (66.66%) and 6 males (33.33%) treated with DMARDs (methotrexate (n=12), hydroxyl chloroquine (n=10), leflunomide (n=8) and low dose (5 mg) Prednisone (n= 11). Their descriptive data were expressed in (Table 1). They were followed up for one year

RF was positive in 14 (66.6%) of patients, while in 12 (60%) of controls. Fourteen patients had extra articular manifestations in the form of subcutaneous nodules (50%), Raynauds phenomena (30%), dry eyes (20%),and carpal tunnel syndrome (10%). Among patients group, DEXA revealed that 7 were osteoporotic (grade 2), 13 were severely osteoporotic (grade 3) while 1 patient was osteopenic (grade 1). Among controls, DEXA revealed that 6 were osteopenic (grade 1), 10 osteoporotic (grade 2) and 4 patients were severely osteoporotic (grade 3).

Before starting anti TNFα therapy when comparing patients versus controls, we found significant increase in age, disease duration, number of TJC, DEXA T scores, serum IL-34 and Cathepsin-K levels towards patients side (Z = -2.68, p= 0.007, Z= -4.291, p= 0,001, Z= -2.53, p= 0.011, Z= -2.291, p=0.022, Z= -4.241, p= 0.001, Z= -4.241, p=0.001) respectively.

Comparison of all clinical laboratories and radiological data of patient's pre versus post treatment to evaluate the effect of anti TNFα therapy was expressed in (Table 2).

The comparison revealed that one year post treatment, patients had highly significant lower ESR, TJC and SJC (p<0.001) than baseline values.

Also There was a statistical significant reduction (P< 0.05) regarding pain, CRP, DAS28, DEXA T-score, serum IL34 level and Cathepsin K level, while minimal increase in SHS . On the other hand, no significant change in s.Il-34, Cathepsin-K, DAS28 and DEXA scores in controls (p > 0.05).

Type of anti TNF α drug

Comparison between patients received etanercept (Enbrel) (n=14) and patients received adalimumab (Humira) (n=7) revealed no statistically significant difference (p>0.05) in any variable between them.

We found that the reduction in morning stiffness, SJC, pain score, DAS 28, serum IL-34 and Cathepsin-K was significantly higher in patients than in controls ((p<0.001). Moreover, the radiographic progression in patients was significantly less than in controls [0.067(0-0.152) vs. [0.159 (0.107- 0.17] (p<0.05) On the other hand, an improvement in DEXA T score was noticed in patients (-0.125 (-0.27–0) while a deterioration in controls (+0.133 (0.053–0.23) (Table 3).

The most sensitive and specific cut off value for IL-34 as a diagnostic test of RA with high disease activity is 375 ng/L with accuracy of 96.7%

Data	Patients (n=21) Median ($25^{th.}$–$75^{th.}$ percentiles)	Controls (n=20) Median ($25^{th.}$–$75^{th.}$ percentiles)
Age(years)	45 (39–50)	39 (33.5–41)
Disease Duration(years)	13 (10.5–20)	3 (2.5–5)
Morning stiffness (hr)	2 (1.5–2.25)	2 (1.5–2.5)
Pain by VAS(cm)	9.5 (9–10)	9 (9–9.5)
MHAQ	1.77 (1.3–2.25)	1.8 (1.125–1.925)
ESR(mm)	50 (45–54)	45 (40–52)
CRP(mg/dl)	30 (18–50)	44 (32–48)
Hb level (g/dl)	11.5 (10.55–12)	11.3 (10.95–12.35)
TJC	20 (18–26)	16 (16–18)
SJC	10 (8–13)	10 (8–12)
DAS28 –CRP	6.54 (5.925–6.73)	5.22 (4.81–5.345)
DEXA T score	-2.8 (-3.5–-1.9)	-1.9 (-2.25–-1.5)
SHS (0-314)	87 (62.5–115)	66 (54.5–79.5)
IL-34 level (ng/L)	950 (740–1250)	125 (125–375)
Cathepsin K level (ng/L)	5.5 (3.75–6.625)	1.5 (0.75–1.75)

Table 1: Descriptive Data of Cases and controls. MHAQ: modified health assessment questionnaire. TJC: tender joint count. SJC: swollen joint count. DAS 28-CRP: disease activity score 28 with CRP. SHS: modified Van Der Heide Sharp score.

Data	Pre ttt	Post ttt	Z	p	Sig.
Morning stiffness	2 (1.5–2.25)	1 (0.5–1.625)	-0.687	0.492	NS
Pain	9.5 (9–10)	7 (4–7.5)	-2.565	0.01	**S**
MHAQ	1.77 (1.3–2.25)	1.25 (1–2.12)	-0.296	0.767	NS
ESR	50 (45–54)	30 (25–38.5)	-2.677	0.007	**HS**
CRP	30 (18–50)	12 (6–17)	-2.524	0.012	**S**
Hb level	11.5 (10.55–12)	12 (11–13)	-1.127	0.26	NS
TJC	20 (18–26)	12 (9–14)	-2.762	0.006	**HS**
SJC	10 (8–13)	6 (2–8)	-2.687	0.007	**HS**
DAS28	6.54 (5.925–6.73)	4.86 (4.38–5.29)	-2.521	0.012	**S**
DEXA scan	-2.8 (-3.5–-1.9)	-2.5 (-3.5–-1.3)	-2.536	0.011	**S**
SHS	87 (62.5–115)	90 (68.5–132)	-2.670	0.008	**HS**
s.IL34 (ng/L	950 (740–1250)	730 (500–837.5)	-2.070	0.038	**S**
Cathepsin K l(ng/L)	5.5 (3.75–6.625)	3.75 (3.125–6)	-2.121	0.034	**S**

Table 2: Comparison of patients characteristics before and after ant TNF α treatment. Pre ttt: pre treatment, post ttt: post treatment patients group data Pain, ESR, CRP, TJC, SJC, DAS 28.,SHS,DEXA scan. IL34, cathepsin K were significantly decreased post treatment.

Variable	Patients	Controls	Z	P	Sig
Δ MS	-0.5(-0.667−-0.354)	0 (-0.225−0)	-3.362	0.001	**HS**
Δ Pain	-0.3(-0.528−-0.211)	-0.222 (-0.236−-0.111)	-2.282	0.022	**S**
Δ MHAQ	-0.111 (-0.355−-0.037)	-0.189 (-0.245−0.112)	-0.385	0.7	NS
Δ ESR	-0.378 (-0.481−-0.245)	-0.25 (-0.315−-0.213)	-1.91	0.056	NS
ΔCRP	-0.556 (-0.75−-0.417)	-0.5 (-0.531−-0.188)	-1.548	0.122	NS
Δ Hb level	0.103 (0.009−0.131)	0.028 (-0.013−0.047)	-1.7	0.089	NS
Δ TJC	-0.444 (-0.538−-0.3)	-0.333 (-0.354−-0.25)	-1.482	0.138	NS
Δ SJC	-0.438 (-0.75−-0.333)	-0.25 (-0.388−-0.208)	-2.749	0.006	**HS**
Δ DAS28	-0.253 (-0.268−-0.157)	-0.121 (-0.193−-0.068)	-2.196	0.028	**S**
Δ DEXA score	-0.125 (-0.27−0)	0.133 (0.053−0.23)	-3.94	0	**HS**
Δ SHS	0.067 (0−0.152)	0.159 (0.107−0.17)	-1.977	0.048	**S**
ΔIL-34 (ng/L)	-0.315 (-0.355−0)	0.333 (0−0.4)	-3.248	0.001	**HS**
Δ Cathepsin K (ng/L)	-0.25 (-0.354−-0.033)	0.167 (0−0.304)	-3.212	0.001	**HS**

Table 3: Comparison between patients and controls regarding change in variables (Δ) before and after treatment. ΔMS: change in morning stiffness. This table showed a significant difference in Δ MS, Δpain, ΔSJC, ΔDAS 28, ΔDEXA score, ΔSHS, ΔIL34 and Δ cathepsin K between patients and controls.

Cut off value	Test of accuracy	Sensitivity	Specificity.		
				PPV	NPV
IL-34 At titre 375 ng/L	96.7 %	95.2 %	100.0 %	100 %	90 %

Table 4: Diagnostic Validity Test of IL-34 for highly active R. PPV: positive predictive value, NPV: negative predictive value.

Cut off value	Test of accuracy	Sensitivity	Specificity	PPV	NPV
Cathepsin K At titre 2 ng/L	90 %	90 %	88.9 %	95.0 %	80 %

Table 5: Diagnostic Validity Test of cathepsin K for RA with osteoporosis.

(Table 4). The most sensitive and specific cut off value for cathepsin K as a diagnostic test of RA with osteoporosis is 2 ng/L. with accuracy of 90% (Table 5). Further analysis of our results to find any association of serum IL-34 with patients' characteristics revealed that baseline Il-34 was positively correlated with baseline disease duration, ESR, CRP and MHAQ (r = 0.852, 0.714, 0.432, 0.685 P< 0.05) respectively and with SJC, DAS28 and SHS (r = 0.515, 0.490, 0.421, P< 0.05) respectively after treatment. Also the change in serum IL-34 after treatment was negatively correlated with DEXA T scores (r = -0.433, p< 0.05).

Similarly, Correlation of baseline serum Cathepsin K with other parameters revealed a positive correlation with SJC, DAS28, SHS and DEXAT scores (r = 0.774, 0.503, 0.711, 0.892, P < 0.05) respectively.

Relation of IL34 cytokine to cathepsin K enzyme

Correlation of Δ IL−34 with Δ Cathepsin K: There was a statistical significant positive correlation (r = 0.523, P< 0.05) between change in serum IL−34 level and change in serum Cathepsin K level throughout the study.

Response according to WHO/ILAR response criteria of improvement

Further analysis of the results revealed that 15 patients were improved (good responders) according to WHO/ILAR response criteria of improvement [53]:

1. >20% improvement in swollen joint count
2. >20% improvement in tender joint count, or > 5 if the count is between 16 and 20
3. >20% improvement in at least two of the following three measures: (i) patient's or physician's global disease activity; (ii) pain; (iii) erythrocyte sedimentation rate. while 6 patients weren't (poor responders) at the end of the study. Comparison between the 2 subgroups regarding clinical, laboratories and radiological variables was done (Table 6).

Relation of Il-34 and cathepsin k to DEXA grading

Krustal Wallis test was used to compare serum IL-34 levels after treatment among the three DEXA subgroups (osteopenic, osteoporotic, severe osteoporotic) and revealed a significant difference between the 3 subgroups (H= 8.795, p = 0.012) as well as significant difference between osteopenic and osteoporotic grades (Z=-2.12, p= 0.034).

Similarly comparison of serum cathepsin K levels after treatment among all grades of DEXA was studied and revealed high significant difference between the 3 grades (H =14.362, p =0.002) (Table 7).

The target of this study was to measure serum levels of IL-34 and Cathepsin K in active long standing RA patients before and after treatment with anti TNF α drugs vs. early RA patients on conventional DMARDS and to evaluate the effect of anti TNF α drugs on disease progression (joint destruction and osteoporosis) in RA patients, so effect of biologic therapy on radiological progression (Δ SHS/year) was measured across treatment time. After one year period of follow up the median (Δ SHS/year) was 0.067 (0- 0.152) which is less than one unit that considered no progression according to previously used definition.

Predictors of radiologic change

To search for predictors that can predict the less radiographic change, by using logistic stepwise multi-regression analysis in 3 models, we get the most sensitive ones which was high baseline DAS 28 and using TNF α inhibitors (Table 8).

Third model showed that high baseline DAS28 and using TNF α inhibitors were the most sensitive predictors for radiologic change.

Variable	Good Responders Median(25-75)	Poor Responders Median(25-75)	Z	P	Sig
Age	42(39-50)	45.5(44.5- 53.75	-1.331	0.183	NS
Disease duration	13(10-23)	13.5(11.75 – 20	-0.431	0.666	NS
MS*	0.75(0.5-1)	2(1.25- 5.625	-2.678	0.007	HS
Pain(VAS)	6 (4-7)	7(6.375-8.25	-1.424	0.155	NS
TJC	12(8-14)	14(10-20)	-1.504	0.133	NS
SJC*	4(1-8)	8(7.5- 9)	-2.315	0.021	S
CRP*	12(6- 12	18 (10.5- 28.5)	-2.132	0.043	S
DAS 28*	4.7 (4.21-4.98)	5.35(4.8975- 6.14	-2.315	0.021	S
SHS	90 (68-124)	89.5(71.25- 165)	-0.624	0.533	NS
DEXA	-2.5(-3.4 -1.2	-2.65 (-3.75-1.817)	-0.546	0.585	NS
HAQ(DI)	1.25(1- 2.12	2.05 (0.89- 2.25)	-0.665	0.506	NS
Cathepsin K	3.75(3- 5.5)	5.25(3.18-10.12)	-1.133	0.257	NS
IL-34*	520(500-770)	937(760-1187.5)	-2.787	0.005	HS

Table 6: Comparison between good and bad responders. This table showed a significant difference in morning stiffness, SJC, DAS 28, and serum IL-34 in good responders versus poor responders.

DEXA grades	Cathepsin K		
Grade 1	Z	P	Sig
Grade 2	14.362	0.002	HS
Grade 3			

Table 7: Comparison of serum Cathepsin K among DEXA grades. This table showed a high significant difference between the 3 grades of DEXA.

Model 1	Reg,Coef.	T	P	Sig	F-Ratio	p	Sig
Constant	-3.175	-1.646	0.124	NS			
Age	0.011	0.563	0.583	NS			
Dis Dur	-0.011	-0.407	0.691	NS			
CRP B	-0.006	-1.099	0.292	NS			
DAS 28 B	0.557	2.125	0.053	NS			
IL-34 B	0	-0.454	0.658	NS			
Cathepsin K	-0.009	-0.128	0.9	NS			
Biologic drug	0.27	1.022	0.325	NS	1.15	0.392	NS

Model 2	Reg. Coef.	T	p	Sig.	F-Ratio	p	Sig
Constant	-2.344	-2.151	0.046	S			
CRP B	-0.005	-1.296	0.212	NS			
DAS28 B	0.427	2.306	0.034	S	2.923	0.064	NS
Biolgic drug	0.297	1.424	0.173	NS			

Model 3	Reg. Coef.	T	p	Sig.	F-ratio	p	Sig
Constant	-1.942	-1.825	0.085	NS			
DAS28 B	0.334	1.922	0.071	NS	3.414	0.045	S
Biologic drug	0.276	1.304	0.209	NS			

Table 8: Stepwise multiple linear regression analysis for ΔSHS. These 3 Models showed that high baseline DAS28 and using TNF α inhibitors were the most sensitive predictors for radiologic progression.

Stepwise multiple linear regression analysis for Δ DEXA was done and showed that high baseline CRP and using TNF α inhibitors were the most sensitive predictors for Δ DEXA and F ratio was 3.954, p=0.038.

Discussion

Rheumatoid arthritis is characterized by subchondral bone loss and the mechanism of bone loss is similar or equal to that of osteoporosis [32]. High BMD loss in RA patients was associated with joint damage progression, disease activity, functional disability and immobility in previous longitudinal studies, even in early RA [33]. Interleukin 34 (IL-34), a recently discovered cytokine that binds macrophage colony-stimulating factor (M-CSF) receptor [34]. Several recent studies revealed that IL-34 expression increases in the synovium, sera, and SFs from patients with RA as well as an association between IL-34 levels and RF and anticyclic citrullinated peptide (CCP) antibody titers [11,34]. It has been known that CSF-1 is present in synovial fibroblasts, plasma, and SF from patients with RA, and that CSF-1R is upregulated in RA synovium [15]. In addition, the administration of anti-CSF-1R antibody reduces the severity of collagen induced arthritis (CIA). After all, it was considered that IL-34 could play a significant role in the synovial inflammation of RA [35]. Functionally, isolated RA-derived fibroblast-like synoviocytes and osteoblasts were found to produce IL-34 in response to TNFα [36]. It was reported that anti TNF-α induced down-regulation of membrane RANKL could be important in preventing articular damage in RA patients. Joint damage may also be mediated by other mediators involved in osteoclast functions such as cathepsin-K [37]. Moreover, the osteoclastogenesis factor RANKL, appears to directly up-regulate cathepsin K expression [38]. Interestingly In the synovium of RA, the cathepsin K protein was localized in synovial fibroblasts, stromal multinucleated giant cells and $CD68^+$ macrophage-like synoviocytes. The overexpression of cathepsin K in RA synovia proves that this protease may become a new and highly specific biomarker for RA.

As previous studies mentioned the pro-osteoclastogenic role of IL-34 and its response to pro-inflammatory cytokines such as tumor necrosis factor-α [39]. Accordingly we suggested that IL-34 can be down regulated under anti TNF-α treatment in human RA, especially in the aspect of joint destruction.

To our knowledge this is the first study for investigating serum levels of both IL34 and cathepsin K as inflammatory and osteoclastogenic factors in early and established RA, before and after anti TNFα therapy versus conventional DMARDS in relation to localized (Joint destruction) and generalized osteoporosis.

We found a significant higher serum IL-34 and Cathepsin-K level in patients with established RA who will receive a biologic therapy than in those with early RA (controls). This could be explained by the difference in disease stage and severity as there was a significant increase in age, disease duration, number of tender joint count, DEXA scores more in patients group than in controls.

Batmaz et al. found that cathepsin K levels were significantly higher in patients with post menopause RA when compared with that of the post menopause healthy control group ($p < 0.05$). In addition, the elevated serum levels of cathepsin K were positively correlated with disease activity score (DAS 28) and total Larsen scores (hands, feet and total) ($p<0.05$) [40]. In addition, Skoumal et al., noticed that cathepsin K serum levels of the patients with RA were significantly elevated (P=0.0003) compared with the healthy control group and a statistically significant correlation between cathepsin K and the Larsen score (P=0.004) [41].

The effect of anti-TNFα therapy was clear by the significant decrease in symptoms and signs of synovitis (pain, TJC, SJC) and activity (ESR, CRP, DAS28-CRP) along with s. IL-34 and s.cathepsin K, and improvement in DEXA T scores at the end of the study in patients group while the reduction in s.IL-34, cathepsin-K, and DAS28 didn't reach significant level ($p>0.05$) in controls group and DEXA scores were deteriorated. This was in accordance with Tian et al, who reported that the level of serum IL-34 decreased after anti-TNF treatment in RA patients [14]. TNF inhibitors delay appearance of bone erosion in RA

patients with no progression of bone destruction in responders and possibly to some extent, in non- responders [42] and Prevention of bone loss [43,44].

On the other hand, our findings of reduction in cathepsin k level post treatment was in contrary with Cauli et al., who reported a persistent increase in cathepsin-K in RA patients under adalimumab (ADA) up to 24 weeks [37]. This may be due to shorter duration of treatment (6 months) than our duration (12 months) and trying two TNFα inhibitors drugs working by two different mechanisms of action: Etanercept (as a soluble receptor antagonist) and ADA (as a monoclonal antibody to TNF-α), therefore, this could successfully decrease cathepsin k level along the study time, reduce and systemic osteoporosis as evident by DEXA T scores were improved in patients post biologic therapy. Moreover, Cauli et al., reported that the inhibition of joint damage and osteoporosis seen in patients treated with anti-TNF-α drugs could be due to reduction in other contributors (DKK-1, sclerostin) in RA rather than reduction in cathepsin k [37]. Moreover, other MMPs and cathepsins were known as potential contributors to bone destruction [45].

Furthermore, although most of patients were improved under biologic therapy, there was no significant difference between patients received Etanercept vs. those received ADA, this may be due to the equal efficacy of both drugs. Our findings are similar to Scott and Kingsley, stated that the TNF inhibitors have exhibited a superior ability to reduce the signs and symptoms of RA, inhibit progression of structural damage and improve physical function in patients with this disease. But the question as to which of the TNF inhibitors affords the greatest efficacy cannot be answered and the choice of agent therefore depends on other factors, including patients' convenience, access to treatment and cost [46].

In our study, to elaborate the effect of anti TNF-α versus DMARDs it was obvious that change in radiologic joint progression per year (ΔSHS) was significantly less in patients than in controls, moreover, a significant difference in DEXA-T scores was noticed reflecting improvement of osteoporosis in patients [-0.125 (-0.27–0)] while deterioration in controls [0.133 (0.053–0.23)] although baseline T scores were higher in patients versus controls. Additionally, Δ IL-34 and Δ cathepsin k were higher in patients under anti TNFα therapy than in controls under conventional DMARDS (Table 3). Vis et al., found that no bone loss was observed in the spine and hip, while a decrease of BMD was observed at the hands after 1 year of infliximab therapy in 102 RA patients [47]. This was in agreement with Marotte et al., who found a significant decrease of BMD (-3.4% at the femoral neck and -3.9% at lumbar spine, $p < 0.001$) in active RA patients treated with methotrexate alone while no decrease was observed in the group treated by infliximab and methotrexate [43].

Marotte and Miossec, concluded that TNFα blockade is not only able to prevent joint destruction, but it is also able to prevent bone loss in RA patients [48].

It was clear that IL-34 is strongly associated with disease activity and radiologic progression as IL-34 was positively correlated with morning stiffness, SHS, ESR and CRP ($p< 0.05$). This was in accordance with a recent study by Chang et al. that baseline IL-34 levels were positively correlated to ΔSHS/year [49]. As it was previously confirmed that SHS was positively correlated with disease activity and local inflammation and this was similar to our findings [50]. Similarly, we found that serum cathepsin k before and after treatment was strongly correlated with TJC, SJC, DAS28, SHS and DEXA T scores which confirms the imperative role of cathepsin k in pathogenesis of RA in both synovitis and joint destruction as well as generalized osteoporosis. This was in accordance with Skoumal et al., proved that cathepsin K is upregulated in the serum and synovial fluid of RA patients with bone degradation. Moreover, its serum concentrations significantly correlated with radiological joint destruction in RA patients [41].

Moreover, a positive correlation ($P<0.05$) between change in serum IL−34 level and change in serum Cathepsin K level throughout the study; and both of them were significantly correlated with DEXA T scores ($p<0.05$), strongly reflects the involvement of both novel markers in disease activity and severity as well as the intimate link between cathepsin k and IL-34 as both of two mediators expressed and exerted joint destruction and osteoporosis in RA through RANKL [38,10]. Morko et al., mentioned that overexpression of cathepsin K leads to spontaneous synovitis and cartilage erosion in RA [51].

After classification the patients under anti TNF α therapy into good and poor responders subgroups, we found significant reduction of morning stiffness, SJC, CRP, DAS28 and serum IL-34 in good responders versus poor responders ($p<0.01$). Similarly further reduction in serum cathepsin k was found in good responders [3.75(3-5.5)] versus poor responders [5.25(3.18-10.12)] which reflected that impact of treatment response was variable on patients of the same group. It may be due to other contributing poor factors as RF positivity, age, gender and disease duration, as three of them were male and all were seropositive and their disease duration range (12-20) years which declare that pathogenesis of RA may differ from a patient to another (disease severity), as well as effect of treatment varied also from one to another patient and this explanation was previously established by many authors [52].

Consequently, in our study, an obvious association was obvious between circulating IL-34 and generalized osteoporosis as a significant difference existed post treatment between s.IL-34 and the three grades of DEXA T scores ($H= 8.795$, $p = 0.012$) and even between any two grades of DEXA T scores. This indicated the potential role of IL-34 in osteoclastogenesis. However, this was in contrary to Moon et al., who reported that no significant correlation was found between serum IL-34 and systemic osteoporosis although IL-34 concentration in synovial fluid was significantly correlated with RANKL levels in his study [34]. On the other hand, our findings for such correlation could be explained by the theory that IL-34 promotes the formation of macrophage colonies from human bone marrow equivalently to CSF-1 and was found to be able to substitute for CSF-1 in receptor activator of nuclear factor kappa-B ligand (RANKL)-induced osteoclastogenesis 3 and 4 [10].

Similarly, when comparing serum level of cathepsin k between subgroups of DEXA T scores, a significant difference existed between them and even between grades of T scores, which reflects that cathepsin k is highly relevant to osteoporosis, with intimate relation to minimal change in bone mineral density, this was clear in our study as cathepsin k had a 90% sensitivity and accuracy to detect RA with osteoporosis at a cutoff value 2 ng/L. Whereas some of the non-osteoclast cell types exhibiting cathepsin K expression open a window of opportunity to consider cathepsin K as a novel target for other disease (chondrocytes: osteoarthritis; synovial fibroblasts: rheumatoid arthritis; macrophages/giant multinucleated cells: atherosclerosis), other sites of expression may raise concern [32].

Conclusion

We found that serum IL 34 and cathepsin k were upregulated in peripheral blood of longstanding RA versus early RA patients. Both were strongly linked to disease activity and were highly relevant to both localized osteoporosis in the form of joint destruction and generalized osteoporosis in the form of DEXA T scores. Furthermore, anti TNF-α therapy effectively decreased both biomarkers regardless drug type, with amelioration of clinical, laboratory and radiological parameters of RA patients.

Acknowledgement

All authors declare that there was no conflict of interest, no funding sources in preparing this work.

References

1. Klareskog L, Catrina AI, Paget S (2009) Rheumatoid arthritis. Lancet 373: 659-672.
2. Upchurch KS, Kay J (2012) Evolution of treatment for rheumatoid arthritis. Rheumatology (Oxford) 51: vi28-36.
3. Takayanagi H (2007) Osteoimmunology: shared mechanisms and crosstalk between the immune and bone systems. Nat Rev Immunol 7: 292-304.
4. Redlich K, Hayer S, Ricci R, David JP, Tohidast-Akrad M, et al. (2002) Osteoclasts are essential for TNF-alpha-mediated joint destruction. J Clin Invest 110: 1419-1427.
5. Schwarz EM, Looney RJ, Drissi MH, O'Keefe RJ, Boyce BF, et al. (2006) Autoimmunity and bone. Ann N Y Acad Sci 1068: 275-283.
6. Schett G (2008) Review: Immune cells and mediators of inflammatory arthritis. Autoimmunity 41: 224-229.
7. Lin H, Lee E, Hestir K, Leo C, Huang M, et al. (2008) Discovery of a cytokine and its receptor by functional screening of the extracellular proteome. Science 320: 807-811.
8. Droin N, Solary E (2010) Editorial: CSF1R, CSF-1, and IL-34, a "menage a trois" conserved across vertebrates. J Leukoc Biol 87: 745-747.
9. Chihara T, Suzu S, Hassan R, Chutiwitoonchai N, Hiyoshi M, et al. (2010) IL-34 and M-CSF share the receptor Fms but are not identical in biological activity and signal activation. Cell Death Differ 17: 1917-1927.
10. Baud'huin M, Renault R, Charrier C, Riet A, Moreau A, et al. (2010) Interleukin-34 is expressed by giant cell tumours of bone and plays a key role in RANKL-induced osteoclastogenesis. J Pathol 221: 77-86.
11. Chemel M, Le Goff B, Brion R, Cozic C, Berreur M, et al. (2012) Interleukin 34 expression is associated with synovitis severity in rheumatoid arthritis patients. Ann Rheum Dis 71: 150-154.
12. Eda H, Zhang J, Keith RH, Michener M, Beidler DR, et al. (2010) Macrophage-colony stimulating factor and interleukin-34 induce chemokines in human whole blood. Cytokine 52: 215-220.
13. Chen Z, Buki K, Vääräniemi J, Gu G, Väänänen HK (2011) The critical role of IL-34 in osteoclastogenesis. PLoS One 6: e18689.
14. Tian Y, Shen H, Xia L, Lu J (2013) Elevated serum and synovial fluid levels of interleukin-34 in rheumatoid arthritis: possible association with disease progression via interleukin-17 production. J Interferon Cytokine Res 33: 398-401.
15. Paniagua RT, Chang A, Mariano MM, Stein EA, Wang Q, et al. (2010) c-Fms-mediated differentiation and priming of monocyte lineage cells play a central role in autoimmune arthritis. Arthritis Res Ther 12: R32.
16. Bradley JR (2008) TNF-mediated inflammatory disease. J Pathol 214: 149-160.
17. Okada Y (2001) Proteinases and matrix degradation. Kelley's Textbook of Rheumatology 6th ed, WB Saunders, Philadelphia, USA.
18. Hou WS, Li Z, Gordon RE, Chan K, Klein MJ, et al. (2001) Cathepsin k is a critical protease in synovial fibroblast-mediated collagen degradation. Am J Pathol 159: 2167-2177.
19. Leung P, Pickarski M, Zhuo Y, Masarachia PJ, Duong LT (2011) The effects of the cathepsin K inhibitor odanacatib on osteoclastic bone resorption and vesicular trafficking. Bone 49: 623-635.
20. Hou WS, Li W, Keyszer G, Weber E, Levy R, et al. (2002) Comparison of cathepsins K and S expression within the rheumatoid and osteoarthritic synovium. Arthritis Rheum 46: 663-674.
21. Svelander L, Erlandsson-Harris H, Astner L, Grabowska U, Klareskog L, et al. (2009) Inhibition of cathepsin K reduces bone erosion, cartilage degradation and inflammation evoked by collagen-induced arthritis in mice. Eur J Pharmacol 613: 155-162.
22. Hou WS, Li Z, Büttner FH, Bartnik E, Brömme D (2003) Cleavage site specificity of cathepsin K toward cartilage proteoglycans and protease complex formation. Biol Chem 384: 891-897.
23. Fuller K, Lawrence KM, Ross JL, Grabowska UB, Shiroo M, et al. (2008) Cathepsin K inhibitors prevent matrix-derived growth factor degradation by human osteoclasts. Bone 42: 200-211.
24. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, et al. (1988): The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 31: 315-324.
25. Aletaha D, Neogi T, Silman A, Funovits J, Felson D, et al. (2010) The 2010 American College of Rheumatology/European League Against Rheumatism Classification Criteria for Rheumatoid Arthritis. Ann Rheum Dis 69: 1580-1588.
26. Fries JF, Spitz P, Kraines RG, Holman HR (1980) Measurement of patient outcome in arthritis. Arthritis Rheum 23: 137-145.
27. Prevoo ML, van 't Hof MA, Kuper HH, van Leeuwen MA, van de Putte LB, et al. (1995) Modified disease activity scores that include twenty-eight-joint counts. Development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. Arthritis Rheum 38: 44-48.
28. Aletaha D, Ward MM, Machold KP, Nell VP, Stamm T, et al. (2005) Remission and active disease in rheumatoid arthritis: defining criteria for disease activity states. Arthritis Rheum 52: 2625-2636.
29. Sharp JT, Young DY, Bluhm GB, Brook A, Brower AC, et al. (1985) How many joints in the hands and wrists should be included in a score of radiologic abnormalities used to assess rheumatoid arthritis? Arthritis Rheum 28: 1326-1335.
30. Grandaunet B, Syversen SW, Hoff M, Sundan A, Haugeberg G, et al. (2011) Association between high plasma levels of hepatocyte growth factor and progression of radiographic damage in the joints of patients with rheumatoid arthritis. Arthritis Rheum 63: 662-669.
31. Kanis JA, Melton LJ 3rd, Christiansen C, Johnston CC, Khaltaev N (1994) The diagnosis of osteoporosis. J Bone Miner Res 9: 1137-1141.
32. Brömme D, Lecaille F (2009) Cathepsin K inhibitors for osteoporosis and potential off-target effects. Expert Opin Investig Drugs 18: 585-600.
33. Jensen T, Klarlund M, Hansen M, Jensen KE, Podenphant J, et al. (2004) Bone loss in unclassified polyarthritis and early rheumatoid arthritis is better detected by digital x ray radiogrammetry than dual x ray absorptiometry: relationship with disease activity and radiographic outcome. Ann Rheum Dis 63:15-22.
34. Moon SJ, Hong YS, Ju JH, Kwok SK, Park SH, et al. (2013) Increased levels of interleukin 34 in serum and synovial fluid are associated with rheumatoid factor and anticyclic citrullinated peptide antibody titers in patients with rheumatoid arthritis. J Rheumatol 40: 1842-1849.
35. Toh Ml, Bonnefoy JY, Accart N, Cochin S, Pohle S, et al. (2014) A CSF-1receptor monoclonal antibody has potent bone and cartilage protective effects in experimental arthritis. Arthritis Rheumatol 66: 2989-3000.
36. Hwang SJ, Choi B, Kang SS, Chang JH, Kim YG, et al. (2012) Interleukin-34 produced by human fibroblast-like synovial cells in rheumatoid arthritis supports osteoclastogenesis. Arthritis Res Ther 14: R14.
37. Cauli A, Alberto A, Dessole G, Grazia P, Porru G, et al. (2012) LIGHT (TNFSF14), Cathepsin-K, DKK-1 and Sclerostin in Rheumatoid Arthritis Patients: Effect of ant TNF- Treatment in the WNT/-Catenin Network Signaling. Arthritis Rheum 64: 498.
38. Fujisaki K, Tanabe N, Suzuki N, Kawato T, Takeichi O, et al. (2007) Receptor activator of NF-kappaB ligand induces the expression of carbonic anhydrase II, cathepsin K, and matrix metalloproteinase-9 in osteoclast precursor RAW264.7 cells. Life Sci 80: 1311-1318.
39. Boström EA, Lundberg P (2013) The newly discovered cytokine IL-34 is

expressed in gingival fibroblasts, shows enhanced expression by pro-inflammatory cytokines, and stimulates osteoclast differentiation. PloS One 8: e81665.

40. Batmaz I, Cakirca G, Sariyildiz MA, Dilek B, Mete N, et al. (2014) Serum osteocalcin, bone alkaline phosphatase and cathepsin k levels of patients with postmenopausal RA: correlation with disease activity and joint damage. Acta Medica Mediterranea 30: 397-401.

41. Skoumal M, Haberhauer G, Kolarz G, Hawa G, Woloszczuk W, et al. (2008) The imbalance between osteoprotegerin and cathepsin K in the serum of patients with longstanding rheumatoid arthritis. Rheumatol Int 28: 637-641.

42. Feldmann M, Maini SR (2008) Role of cytokines in rheumatoid arthritis: an education in pathophysiology and therapeutics. Immunol Rev 223: 7-19.

43. Marotte H, Pallot-Prades B, Grange L, Gaudin P, Alexandre C, et al. (2007) A 1-year case-control study in patients with rheumatoid arthritis indicates prevention of loss of bone mineral density in both responders and nonresponders to infliximab. Arthritis Res Ther 9: R61.

44. Smolen JS, Han C, Bala M, Maini RN, Kalden JR, et al. (2005) Evidence of radiographic benefit of treatment with infliximab plus methotrexate in rheumatoid arthritis patients who had no clinical improvement: a detailed subanalysis of data from the anti-tumor necrosis factor trial in rheumatoid arthritis with concomitant therapy study. Arthritis Rheum 52: 1020–1030.

45. Schurigt U, Hummel KM, Petrow PK, Gajda M, Stöckigt R, et al. (2008) Cathepsin K deficiency partially inhibits, but does not prevent, bone destruction in human tumor necrosis factor-transgenic mice. Arthritis Rheum 58: 422-434.

46. Scott DL, Kingsley GH (2006) Tumor necrosis factor inhibitors for rheumatoid arthritis. N Engl J Med 355: 704-712.

47. Vis M, Havaardsholm EA, Haugeberg G, Uhlig T, Voskuyl AE, et al. (2006) Evaluation of bone mineral density, bone metabolism, osteoprotegerin and receptor activator of the NFkappaB ligand serum levels during treatment with infliximab in patients with rheumatoid arthritis. Ann Rheum Dis 65: 1495-1499.

48. Marotte H, Miossec P (2008) Prevention of bone mineral density loss in patients with rheumatoid arthritis treated with anti-TNFalpha therapy. Biologics 2: 663-669.

49. Chang SH, Choi BY, Choi J, Yoo JJ, Ha YJ, et al. (2015) Baseline serum interleukin-34 levels independently predict radiographic progression in patients with rheumatoid arthritis. Rheumatol Int 35: 71-79.

50. van der Heijde D (2001) Radiographic progression in rheumatoid arthritis: does it reflect outcome? Does it reflect treatment? Ann Rheum Dis 60 Suppl 3: iii47-50.

51. Morko J, Kiviranta R, Joronen K, Säämänen AM, Vuorio E, et al. (2005) Spontaneous development of synovitis and cartilage degeneration in transgenic mice overexpressing cathepsin K. Arthritis Rheum 52: 3713-3717.

52. Arenere Mendoza M, Manero Ruiz FJ, Carrera Lasfuentes P, Navarro Aznarez H, Pecondon Espanol A, et al. (2010) Tumour necrosis factor alpha antagonists in established rheumatoid arthritis: Effectiveness comparative study. Med Clin (Barc) 134: 665-670.

53. Van Riel PL, Van Gestel M, Van De Putte LB (1996) Development and validation of response criteria in Rheumatoid arthritis: steps toward an international consensus on prognostic markers. Br J Rheum 35: 4-7.

Citation: Saber N, Atef M, AbdelAziz D (2015) Joint Destruction and Osteoporosis are Associated with Upregulation of IL-34 and Cathepsin k Expression in Rheumatoid Arthritis. Clinical Trial with Anti TNF α Therapy. J Arthritis 4: 167. doi:10.4172/2167-7921.1000167