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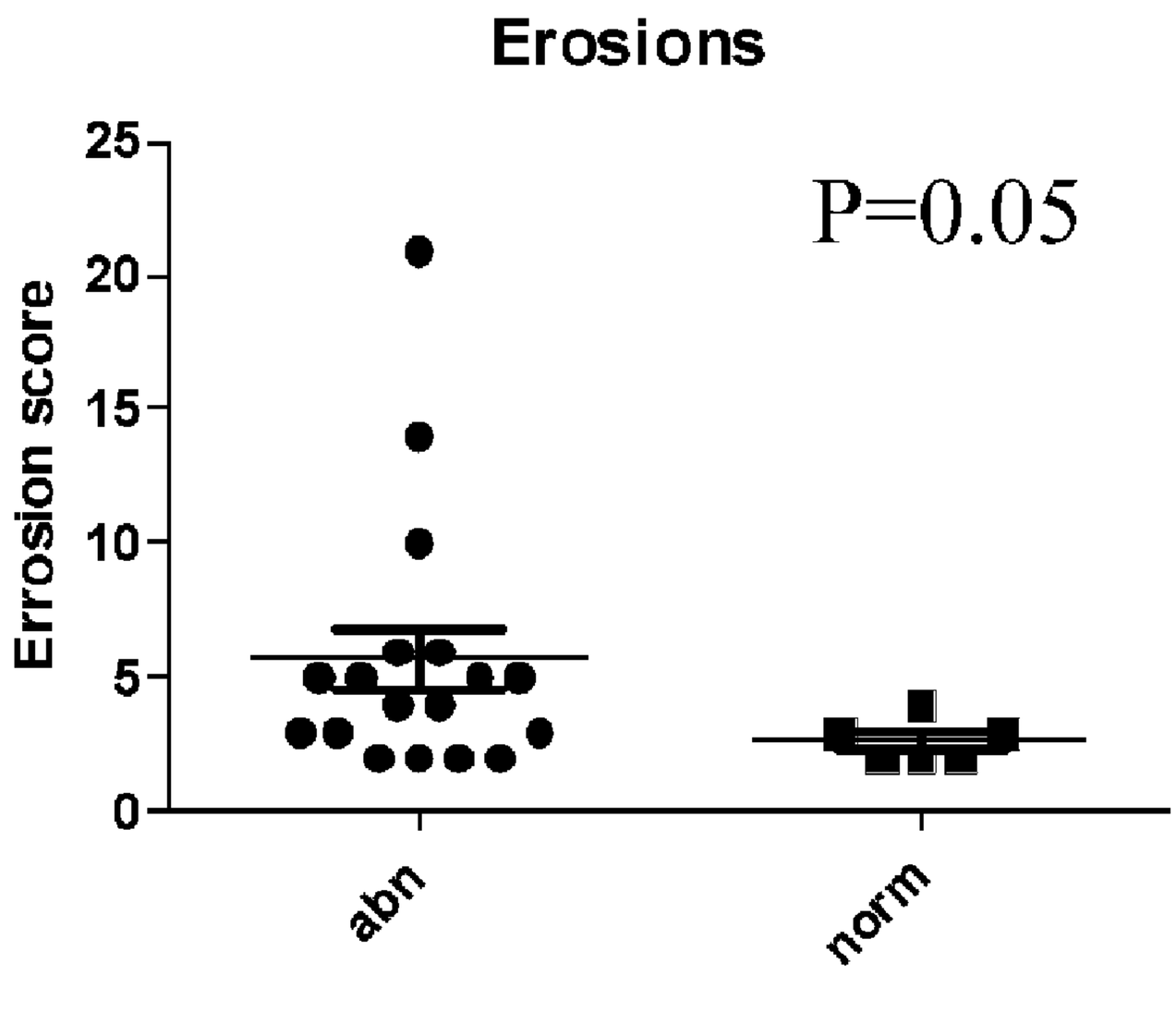

FIGURE 5

(57) Abrégé/Abstract:
The present invention provides a method of determining the rheumatoid arthritis status of a subject, or the progression of rheumatoid arthritis, or the appropriate treatment for a subject with rheumatoid arthritis, comprising the steps of (a) determining the level of tenascin-C in a sample from said subject; and (b) comparing the level of tenascin-C determined in step (a) with one or more reference values. Preferably the rheumatoid arthritis referred to is erosive rheumatoid arthritis. The be accompanied when published by Figure 5.

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[Continued on next page]

(54) Title: TENASCIN-C AND USE THEREOF IN RHEUMATOID ARTHRITIS

(57) Abstract: The present invention provides a method of determining the rheumatoid arthritis status of a subject, or the progression of rheumatoid arthritis, or the appropriate treatment for a subject with rheumatoid arthritis, comprising the steps of (a) determining the level of tenascin-C in a sample from said subject; and (b) comparing the level of tenascin-C determined in step (a) with one or more reference values. Preferably the rheumatoid arthritis referred to is erosive rheumatoid arthritis. The be accompanied when published by Figure 5.

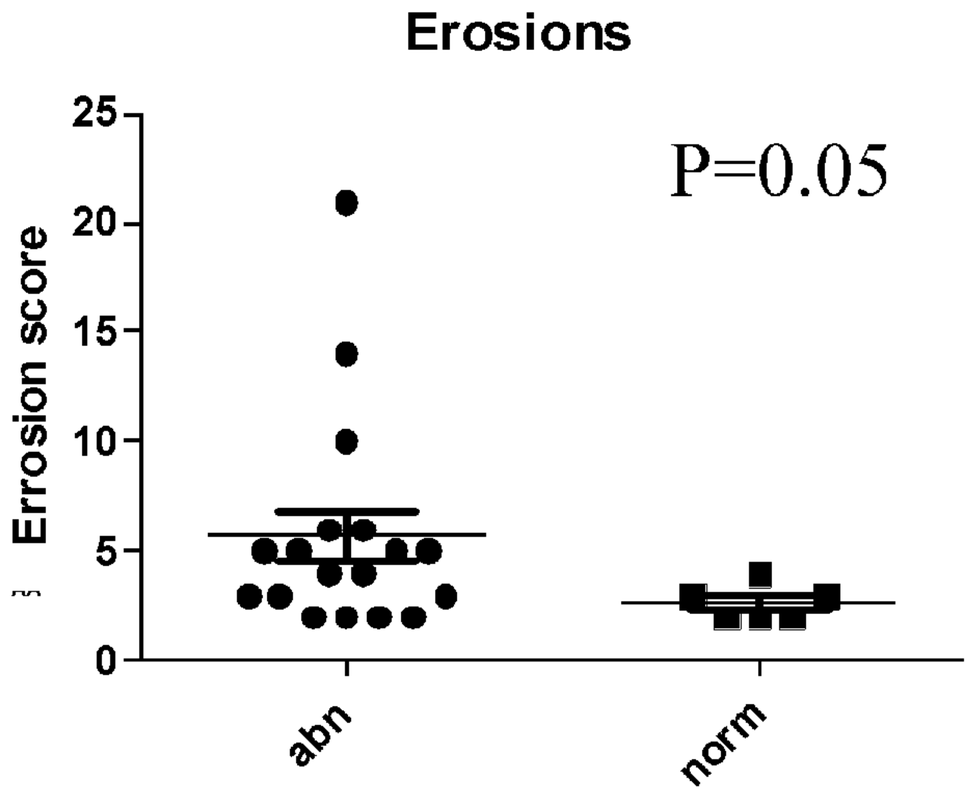

FIGURE 5

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BIOMARKER AND USE THEREOF

The present invention relates to the use of tenascin-C as a biomarker for inflammatory disorders, and in particular as a serum biomarker for rheumatoid arthritis. In particular, the invention relates to the use of tenascin-C as a biomarker for erosive rheumatoid arthritis.

Tenascin-C is a proinflammatory extracellular matrix glycoprotein (Midwood et al (2009) Nat. Med. 15: 774-780), that induces inflammatory cytokine synthesis in primary human macrophages and synovial fibroblasts by activation of the pattern recognition receptor, TLR4. Tenascin-C is a large hexameric protein of 1.5 million Da. High levels of tenascin-C have been found at sites of inflammation, for example in the synovial fluid of rheumatoid arthritis patients and in tumor stroma.

Rheumatoid arthritis is a chronic disease characterized by prolonged inflammation, swelling and pain of multiple joints. With time, the chronic inflammation leads to bone destruction within the joints and to progressive disability. One prominent hallmark of rheumatoid arthritis is wide variability in its clinical presentation. This variability extends to the level of pain, number of swollen joints and extent of joint deformity. Similarly, the response of patients with rheumatoid arthritis to any specific medical therapy also varies widely, from near elimination of disease signs and symptoms in some patients, to almost complete unresponsiveness in others.

The current diagnostic approach for rheumatoid arthritis was established by the American College of Rheumatology and is composed of the following items:

1) morning stiffness lasting more than 1 hour (mainly in fingers);

2) swelling in more than 3 joints;

3) swelling in joints of hand (wrists, metacarpophalangeal joints and proximal interphalangeal joints);

4) swelling in symmetrical joints (right and left joints);

5) abnormal findings of radiography of hand;

6) subcutaneous nodules; and

7) test positive for rheumatism by a blood test for CRP (C-reactive protein) or anti-CCP (anti-cyclic citrullinated peptide). The case which satisfies more than 4 items is diagnosed as rheumatoid arthritis.

It is an object of the present invention to provide an alternative marker, a diagnostic agent, and a detecting method that may be conveniently used for the identification of rheumatoid arthritis, and in particular for the identification of erosive rheumatoid arthritis.

Erosive rheumatoid arthritis is a major cause of disability in patients with rheumatoid arthritis, and is characterised by bone loss and local erosion of articular bone.

Determination of bone erosion is almost exclusively based on radiographic findings, ultrasound and doplar studies, because direct assessment of these lesions through biopsy is only rarely practical. The term 'bone erosion' describes loss of mineralized tissue at juxta-articular sites, which is commonly associated with a break in the cortical lining. Bone erosion starts early in disease and progresses most rapidly during the first year, thus the ability to rapidly detect and treat erosive rheumatoid arthritis would be of great benefit.

According to a first aspect, the invention provides a method of determining the inflammatory disorder status of a subject comprising the steps of:

(a) determining the level of tenascin-C in a sample from said subject; and

(b) comparing the level of tenascin-C determined in step (a) with one or more reference values.

The inflammatory disorder may be one or more of rheumatoid arthritis, and erosive rheumatoid arthritis in particular, ankylosing spondylitis, psoriatic arthritis, lupus and myositis. Preferably the inflammatory disorder is rheumatoid arthritis, and in particular erosive rheumatoid arthritis.

In a preferred embodiment the method is used to determine the rheumatoid arthritis status of a subject, and more preferably the erosive rheumatoid arthritis status of a subject. Erosive rheumatoid arthritis is defined as rheumatoid arthritis with at least one erosive lesion in the articular bone, preferably there are at least five lesions, preferably there are at least ten lesions, preferably there are at least 20 lesions. The higher the number of lesions the more severe the erosive rheumatoid arthritis.

The method of the invention may be used to determine the erosive rheumatoid arthritis status of a subject who has already been diagnosed as having rheumatoid arthritis. Preferably the method of the invention provides an easy, non-invasive and cheap way to look at the erosive rheumatoid arthritis status of a patient with rheumatoid arthritis.

The sample may be a blood sample, such as a whole blood sample, plasma or serum. In an alternative embodiment the sample may be urine. Preferably the sample is a serum sample.

Preferably the method is used to determine whether or not a subject has erosive rheumatoid arthritis.

Tenascin-C is a novel serum biomarker for inflammatory disorders, and rheumatoid arthritis in particular, and more specifically erosive rheumatic arthritis.

The protein sequence of human tenascin C can be found on GenBank using the accession number P24821 and the amino acid sequence is given in Figure 8.

In the method of the invention all forms of tenascin C may be measured. Preferably at least a 320kDa isoform of tenascin C is measured. Preferably only a 320kDa isoform of tenascin C is measured.

The phrase "inflammatory disorder status" includes any distinguishable manifestation of the inflammatory disorder. For example, inflammatory disorder status includes, without limitation, the presence or absence of the inflammatory disorder, the risk of developing the inflammatory disorder, the stage of the inflammatory disorder, the progression of the inflammatory disorder, and the effectiveness or response of a subject to a treatment for the inflammatory disorder.

The method of the invention may be used, for example, for any one or more of the following: to diagnose an inflammatory disorder in a subject; to assess the chance of a subject developing an inflammatory disorder; to advise on the prognosis for a subject with an inflammatory disorder; to monitor disease progression; and to monitor effectiveness or response of a subject to a treatment for an inflammatory disorder.

Preferably the method allows the diagnosis of an inflammatory disorder in a subject from the analysis of the level of tenascin-C in a sample provided by the subject.

The phrase "rheumatoid arthritis status" includes any distinguishable manifestation of the disease. For example, rheumatoid arthritis status includes, without limitation, the presence or absence of rheumatoid arthritis, the risk of developing rheumatoid arthritis, the stage of rheumatoid arthritis, the progression of rheumatoid arthritis, and the effectiveness or response of a subject to a treatment for rheumatoid arthritis. In a preferred embodiment rheumatoid arthritis status refers to the absence or presence of erosive rheumatoid arthritis.

The method of the invention may be used, for example, for any one or more of the following: to diagnose rheumatoid arthritis status in a subject, in particular erosive rheumatoid arthritis; to assess the chance of a subject developing rheumatoid arthritis, and in particular erosive rheumatoid arthritis; to advise on the prognosis for a subject with rheumatoid arthritis, and in particular erosive rheumatoid arthritis; to monitor disease progression, and in particular erosive rheumatoid arthritis progression; and to monitor effectiveness or response of a subject to a treatment for rheumatoid arthritis, and in particular erosive rheumatoid arthritis.

Preferably the method allows the diagnosis of rheumatoid arthritis, and in particular erosive rheumatoid arthritis, in a subject from the analysis of the level of tenascin-C in a sample provided by the subject.

The level of the tenascin-C present in a sample may be determined by any suitable assay, which may comprise the use of any of the group comprising immunoassays, spectrometry, western blot, ELISA, immunoprecipitation, slot or dot blot assay, isoelectric focussing, SDS-PAGE and antibody microarray immunohistological staining, radio immuno assay (RIA), fluoroimmunoassay, an immunoassay using an avidin-biotin or streptoavidin-biotin system, etc or combinations thereof. These methods are well known to persons skilled in the art.

Alternatively, the level of tenascin-C may be measured by determining the tenascin-C mRNA levels in a sample. The mRNA levels could be measured by PCT or any other suitable technique.

Preferably the reference value, to which the determined levels of tenascin-C are compared, is the level of tenascin-C observed in one or more subjects that do not have any detectable inflammatory disorder, such as rheumatoid arthritis, or any clinical symptoms of an inflammatory disorder, such as rheumatoid arthritis, and have so called "normal values" of the biomarker tenascin-C.

Preferably an about 50% or more increase in tenascin-C in a sample, compared to the level in a normal tissue sample, is diagnostic of an inflammatory disorder, for example rheumatoid arthritis, and in particular of erosive rheumatoid arthritis.

Preferably a level of more than 31ng, preferably more than 33ng, of tenascin-C per ml of serum or plasma is diagnostic or at least indicative of rheumatoid arthritis, and in particular of erosive rheumatoid arthritis. Preferably the level of tenascin-C in combination with other markers allows a diagnosis of rheumatoid arthritis, and in particular of erosive rheumatoid arthritis.

Alternatively, the reference value may be a previous value obtained for a specific subject. This kind of reference value may be used if the method is to be used to monitor progression of disease or to monitor the response of a subject to a particular treatment.

Preferably, an increase in the level of tenascin-C compared to a normal/non-diseased reference level is indicative, or diagnostic, of rheumatoid arthritis, and erosive rheumatoid arthritis in particular.

The level of tenascin-C may be used to stratify patients with rheumatoid arthritis to those with erosive rheumatoid arthritis and those without.

The method of the invention may also be used to monitor rheumatoid arthritis progression, and erosive rheumatoid arthritis progression in particular, and/or to monitor the efficacy of treatments administered to a subject. This may be achieved by analysing samples taken from a subject at various time points following initial diagnosis and monitoring the changes in the levels of tenascin-C and comparing these levels to normal and/or reference values. In this case reference levels may include the

initial levels of the biomarker in the subject, or the levels of the biomarker in the subject when they were last tested, or both.

The method of the invention may also be used to determine the appropriate treatment for a subject. The method may be used to offer personalised medicine solutions. In one embodiment, an increased level of tenascin-C in a sample, sufficient to result in a diagnosis of an inflammatory condition such as rheumatoid arthritis, may be used to indicate that it is not appropriate to use an anti-TNF drug as the subject is not likely to respond. It may be more appropriate to give an alternative therapy, such as an anti-IL17 therapy; a T-cell co-stimulation modulator (such as Orencia™ – abatacept): an interleukin-6 (IL-6) inhibitor (such as Actemra™ – tocilizumab); an anti-CD20 antibody (such as Rituxan™ – rituxumab; or a B cell activating factor (such as anti-BAFF). Other alternative therapies include inhibitors of janus kinase (JAK) (such as Tofacitinib™) and inhibitors of spleen tyrosine kinase (Syk) (such as Fostamatinib™).

In one embodiment the invention provides a method for determining whether anti-TNF therapy is an appropriate treatment for a patient with rheumatoid arthritis. More specifically, if a patient with rheumatoid arthritis has a tenascin-C level in their plasma or serum of at least 31ng, or at least 33ng, per ml of plasma or serum then they are unlikely to respond to anti-TNF therapy.

Preferably the method of the invention is carried out *in vitro*.

The subject may be mammal, and is preferably a human, but may alternatively be a monkey, ape, cat, dog, cow, horse, rabbit or rodent.

According to a further aspect the invention provides a method of determining the erosive rheumatoid arthritis status of a subject with rheumatoid arthritis comprising:

(a) determining the level of tenascin-C in a sample from said subject; and

(b) comparing the level of tenascin-C determined in step (a) with one or more reference values.

(c)

Preferably the sample is a serum sample or a plasma sample. Preferably the level of tenascin-C in the sample is at least 31ng, or at least 33ng, per ml of sample. Preferably the reference value is the value of tenascin-C in a plasma or a serum

sample from a subject who does not have rheumatoid arthritis. The skilled man will appreciate that all the preferred features referred to above may also apply to this and every aspect of the invention.

According to another aspect of the invention there is provided a kit for use in determining the inflammatory disorder status of a subject comprising at least one agent for determining the level of tenascin-C in a sample provided by the subject.

In a preferred embodiment the kit is for use in determining the rheumatoid arthritis status, and in particular the erosive rheumatoid arthritis status, of a subject comprising at least one agent for determining the level of tenascin-C in a sample provided by the subject.

The agent may be an antibody.

The kit may comprise instructions for suitable operational parameters in the form of a label or separate insert. The instructions may inform a consumer about how to collect the sample.

The kit may comprise one or more tenascin-C samples to be used as standard(s) for calibration and comparison. The kit may also comprise instructions to compare the level of tenascin-C detected in a sample with a calibration sample or chart. The kit may also include instructions indicating what level of tenascin-C is diagnostic of rheumatoid arthritis, and of erosive rheumatoid arthritis in particular.

According to a yet further aspect, the invention provides the use of the determination of the level of tenascin-C in a blood or serum sample as a means of assessing the inflammatory disorder status in an individual.

In a preferred embodiment the invention provides the use of the determination of the level of tenascin-C in a blood or serum sample as a means of assessing the rheumatoid arthritis status, and in particular the erosive rheumatoid arthritis status, in an individual.

According to another aspect the invention provides a method of treating an inflammatory condition such as, rheumatoid arthritis and in particular erosive rheumatoid arthritis, in a subject comprising determining the level of tenascin-C in a sample from the subject and administering an inflammatory treatment, and in particular a treatment for rheumatoid arthritis or erosive rheumatoid arthritis based on the level of tenascin-C observed. Preferably a treatment is administered if the tenascin-C level is greater than the levels in a reference sample. The reference sample may be a sample from a normal subject who does not have an inflammatory condition. The treatment may be administered if the levels of tenascin-C are greater than 31ng/ml, preferably greater than 33ng/ml. If the level of tenascin-C is greater than 31ng/ml, and preferably greater than 33ng/ml, then an alternative therapy to an anti-TNF therapy may be administered. The alternative therapy may be one of the aforementioned therapies.

According to a further aspect the invention provides a method of treating rheumatoid arthritis, and in particular erosive rheumatoid arthritis, comprising determining the level of tenascin-C in a sample from the subject and administering an anti-TNF therapy if the tenascin-C level is greater than the level in a reference sample but less than 33ng/ml, preferably less than 31ng/ml.

According to a yet further aspect the invention provides a method of treating rheumatoid arthritis, and in particular erosive rheumatoid arthritis, comprising determining the level of tenascin C in a sample from the subject and administering a therapy which is not an anti-TNF therapy if the tenascin C level is greater than 31ng/ml, preferably greater than 33ng/ml.

The alternative therapy may be an anti IL-17 therapy or any of the aforementioned alternative therapies.

The skilled man will appreciate that preferred features of any one embodiment and/or aspect of the invention may be applied to all other embodiments and/or aspects of the invention.

The present invention will be further described in more detail, by way of example only, with reference to the following figures in which:

Figure 1 – illustrates that tenascin-C levels are significantly increased in patients with rheumatoid arthritis. 52 patients were considered in this study, and 86.6% of those with rheumatoid arthritis were shown to have abnormally high (significantly increased) levels of tenascin-C. The results demonstrate that the mean tenascin-C value for rheumatoid arthritis patients is 87.87ng/ml of serum, and for the normal population the mean tenascin-C value is 20.34ng/ml of serum. The 95% confidence limit for the patients with rheumatoid arthritis is 31ng/ml, and thus it is concluded that levels of tenascin-C of above 31ng/ml serum are diagnostic of rheumatoid arthritis.

Figure 2 – is a Western blot showing circulating levels of tenascin C in patients with rheumatoid arthritis (RA). Figure 2A shows that in baseline samples from RA patients one predominant TNC band of 320 kDa was observed (n=18). Corresponding TNC levels detected in the same samples by ELISA are shown in pg/ml under the blot. Low levels of TNC were detected in healthy control plasma (N). rhTNC= 0.05µg human recombinant TNC. Figure 2B illustrates that in addition to the major band detected at 320kDa, bands of MW 219, 201, 190 and 156 kDa were present in the plasma of some RA patients upon western blotting with anti tenascin-C antibodies (TNC). Ponceau S staining of the same membrane shows equivalent protein loading for each RA sample.

Figure 3 – demonstrates that there is no correlation between tenascin-C levels and the levels of currently used CRP and anti-CCP markers for arthritis. Thus demonstrating tenascin-C to be an alternative and improved biomaker, in particular for rheumatoid arthritis, and erosive rheumatoid arthritis specifically. 52 patients were considered in this study, and serum levels of tenascin-C compared to serum levels of CRP and anti-CCP. There was no relationship between levels of TNC with either CRP and anti-CCP indicating that tenascin-C is a unique biomarker of rheumatoid arthritis.

Figure 4 – illustrates that there is a correlation between the level of tenascin-C expression in the serum of a patient with rheumatoid arthritis and the degree of erosion observed in the joints of a patient – the x axis represents the number of bone erosions observed. The data presented shows that the level of tenascin-C

in serum is indicative of the presence of bone erosions in the joint and that the higher the tenascin-C serum levels the more erosion is observed.

Figure 5 – illustrates that when patients with rheumatoid arthritis are divided into those with abnormal levels of tenascin-C in the serum (that is, greater than 31ng/ml) and those with normal levels of tenascin-C (that is with tenascin-C levels in the serum of 0-31ng/ml) the patients with abnormal tenascin-C levels have a statistically increased erosion score. Thus indicating that the tenascin-C level in the serum is a good biomarker for erosive rheumatoid arthritis.

Figures 6A – 6C – demonstrate that there is no correlation between tenascin-C levels and tender and swollen joint count (counted by the GP) (Figure 6A), or with the global assessment score (Physician global assessment. On a scale 1-10 how does the doctor rate the activity of the patients disease) (Figure 6B) or the biochemical marker CRP or with the erythrocyte sedimentation rate (ESR) (Figure 6C). CRP is an acute phase protein that is elevated in inflammation, and is a non-specific maker of inflammation, levels are measured by ELISA. The ESR is the rate at which red blood cells sediment in a period of 1 hour, and is a non-specific measure of inflammation. To perform the test, anticoagulated blood is placed in an upright tube, known as a Westergren tube, and the rate at which the red blood cells fall is measured and reported in mm/h. The ESR is governed by the balance between pro-sedimentation factors, mainly fibrinogen, and those factors resisting sedimentation, namely the negative charge of the erythrocytes (zeta potential). When an inflammatory process is present, the high proportion of fibrinogen in the blood causes red blood cells to stick to each other.

Figure 7– demonstrates the effect of the anti-TNF therapy Infliximab™ and methotrexate (MTX) on the tenascin-C levels in seven patients with rheumatoid arthritis. The results demonstrate that these therapies have very little long term effect on tenascin-C.

Figure 8 – details the amino acid sequence of human tenascin C.

Figure 9 – demonstrates that tenascin-C level at baseline is predictive of future tender joint count in RA patients at both 16-18 weeks and 54 weeks after commencing infliximab (anti-TNF) therapy. Serum from RA patients in cohort A at baseline (entry into the trial) was analysed for tenascin-C level by ELISA. Tenascin-C levels are shown with practitioner determined tender joint counts in these patients at 16-18 weeks (A) and 54 weeks (B) after receiving Infliximab therapy. Significance was determined by spearman rank correlation analysis. Cohort A includes patients with a diagnosis of erosive RA according to the ACR 1987 criteria, with symptoms of 0.5 to 3 years duration. Cohort B includes patients with a diagnosis of RA with disease activity scored at a moderate level or higher.

Assessment of tenascin-C levels in patients with rheumatoid arthritis in comparison with in normal/control subjects

Serum was obtained from the blood of patients with rheumatoid arthritis and from normal subject and stored at -80°C until used. The sera was thawed on ice on the day of analysis and diluted typically 1:50 in EIA buffer (1% BSA, 0.05% Tween 20 in PBS) for use in ELISA. ELISA kits were purchased from IBL (Cat No. 27751) and used to detect the high molecular weight variant of human tenascin-C. 100µl of diluted sera was incubated on ELISA plates pre-coated with non-labelled capture anti-tenascin-C antibody, in duplicate at 37°C for 60 mins. The plates were washed 7 times (in 0.001% Tween 20/PBS) and HRP-labelled anti-human tenascin-C antibody was added (100µl) to each well. The wells were then incubated for 30mins at 4°C, and then washed 9 times with 0.001% Tween 20/PBS. 100µl Chromogen (supplied TMB solution) was then added to each well and incubated in the dark for 30 mins or until colour change judged to be sufficient. The reaction was stopped by the addition of 100µl 1N H_2SO_4. The colour change was read at 450nm using a multiscan plate analyser, and the concentration of tenascin-C was calculated using Ascent version 2.6. Using standard curve generated from the tenascin-C supplied with ELISA plate (24 – 0.38 ng/ml) as a standard.

The above ELISA was performed on serum samples obtained from 52 patients with rheumatoid arthritis and from 20 normal control subjects who showed no signs of rheumatoid arthritis. The results are shown in Figure 1 and demonstrate a

significantly higher level of tenascin-C in the rheumatoid arthritis patients, and allows the conclusion to be drawn that a level of tenascin-C greater than 31ng/ml, or greater than 33ng/ml, of serum is diagnostic of rheumatoid arthritis, and erosive rheumatoid arthritis in particular.

Western blotting revealed that the major form of tenascin-C present in rheumatoid arthritis patient samples has a mass of 320kDa, (Figure 2A) but minor, smaller, forms of tenascin-C were also observed in some patients (Figure 2B).

The Western blot data in Figure 2 was obtained using the following method. 1µl of serum or plasma was electrophoresed on 4-12% Bis-Tris pre-cast polyacrylamide gels (Invitrogen, Life Technologies, Paisley, UK). Proteins were transferred onto nitrocellulose membranes (Amersham, GE Healthcare, Chalfont, UK) and visualised using Ponceau S stain (Sigma Aldrich, Gillingham, UK). After washing with TBS/0.01% Tween to remove stain, membranes were blocked in 5% BSA/TBS-Tween for 1h at room temperature and incubated with primary antibody recognising the N-terminal region of human TNC (MAB 1908, Merck Millipore, Watford UK) at 1:1000 dilution for 1h at 37°C. Horseradish peroxidase-conjugated anti-mouse IgG (Dako, Ely, UK) was used as a secondary antibody at a dilution of 1:3000. Bound antibody was detected using the enhanced chemiluminescence kit (Amersham, GE Healthcare) and visualized on Super RX medical X-ray film (Fuji, Japan

Correlation between tenascin-C levels in patients with rheumatoid arthritis and the level of bone erosion

In order to demonstrate the correlation between the level of tenascin-C in a patient's serum and erosive rheumatoid arthritis, the level of erosion in the rheumatoid arthritis patients was compared to the level of circulating tenascin-C in the serum.

Erosion was measured using ultrasound and power doplar imaging as described by Taylor PC in Rheumatology (Oxford). 2005 Jun;44(6):721-8. Epub 2005 Jan 11. Review.

As can be seen in Figure 4 when the erosion score is plotted against the tenascin-C levels a significant correlation is observed.

Figure 5 goes on to demonstrate an even stronger correlation with erosive rheumatoid arthritis by comparing the erosion score of patients with rheumatoid arthritis who have abnormal levels of tenascin-C in the serum (that is, greater than 31ng/ml) with those of patients with rheumatoid arthritis who have normal levels of tenascin-C (that is with tenascin-C levels in the serum of 0-31ng/ml). The patients with abnormal tenascin-C levels were shown to have a statistically increased erosion score. Thus indicating that tenascin-C levels in the serum is good biomarker of erosive rheumatoid arthritis.

Furthermore, no significant correlation with other measures of rheumatoid arthritis and tenascin-C levels was observed (Figures 6A-6C), further indicating tenascin-C levels in serum to be a good biomarker for rheumatoid arthritis, and in particular for erosive rheumatoid arthritis.

The effect of treatments for rheumatoid arthritis on tenascin-C levels

As illustrated in Figure 7, currently used treatments for rheumatoid arthritis do not appear to have any affect on tenascin-C levels.

High serum tenascin-C is predictive of unresolved joint tenderness in infliximab treated patients

Despite the major advances provided by therapy with anti-TNF agents such as infliximab, a significant proportion of rheumatoid arthritis patients treated with anti-TNF agents do not respond and continue to acquire joint erosions and increased DAS28 scores. In the light of the correlation of tenascin-C levels with erosion scores and the effect of infliximab on circulating tenascin-C levels, studies were undertaken to determine whether the level of tenascin-C served as a predictor of future disease progression in infliximab treated patients. There was no relationship between baseline level of tenascin-C or change in tenascin-C level at weeks 18 or 52 after commencement of therapy and response to infliximab. However, a significant correlation was seen between the level of tenascin-C at baseline and the subsequent

TJC score in infliximab treated patients at both 16-18 and 54 weeks after the start of treatment (Figure 9). Thus, these data suggest that patients with higher levels of tenascin-C before the initiation of infliximab therapy are more likely to be associated with unresolved joint tenderness despite infliximab therapy.

The results indicate that the level of tenascin-C in serum before beginning anti-TNF treatment acts as a predictor of tender joint count in a patient as far as one year in advance and allows patients that are likely to benefit from anti-TNF treatment to be distinguished from those who are not likely to respond.

Furthermore the results presented in Figure 2 indicate that the predominant variant of tenascin-C in the serum of patients with rheumatoid arthritis, and erosive rheumatoid arthritis in particular, is an isoform 320kDa in mass, which constitutes a 'large isoform' possessing. Tenascin-C is a large, multimodular molecule. It comprises a number of distinct domains including an assembly domain, a series of 14 and a half epidermal growth factor-like repeats, a series of up to 17 fibronectin type III-like repeats (TNIII), and a fibrinogen-like globe. Tenascin-C is encoded by a single gene that is alternatively spliced to create monomers ranging in size from 190-320 kDa. This occurs specifically in the TNIII domains; 8 of which are constitutively expressed (TNIII1-8) and 9 of which can be alternatively spliced (A1-4, B, AD2, AD1, C and D).

CLAIMS

1. A method of determining the rheumatoid arthritis status of a subject comprising the steps of:

(a) determining the level of tenascin-C in a sample from said subject; and

(b) comparing the level of tenascin-C determined in step (a) with one or more reference values.

2. A method of determining the progression of rheumatoid arthritis or the response of rheumatoid arthritis to a particular treatment in a subject comprising the steps of:

(a) determining the level of tenascin-C in a sample from said subject; and

(b) comparing the level of tenascin-C determined in step (a) with one or more reference values.

3. A method for determining the appropriate treatment for a subject with rheumatoid arthritis comprising the steps of:

(a) determining the level of tenascin-C in a sample from said subject; and

(b) comparing the level of tenascin-C determined in step (a) with one or more reference values

(c) using the results in (b) to determine the most appropriate therapy.

4. The method of any preceding claim wherein the rheumatoid arthritis is erosive rheumatoid arthritis.

5. The method of claim 1 for use in determining or diagnosing whether a subject with rheumatoid arthritis has erosive rheumatoid arthritis.

6. The method of any preceding claim wherein the sample is a blood sample, such as a whole blood sample, plasma or serum.

7. The method of any preceding claim wherein an about 50% or more increase in tenascin-C in a sample, compared to a reference level, is diagnostic of an inflammatory disorder, for example rheumatoid arthritis, and in particular of erosive rheumatoid arthritis.

8. The method of claim 7 wherein the reference level is the level in a normal sample.

9. The method of any of claims 1 to 7 wherein a level of more than 33 ng of tenascin-C per ml of serum is diagnostic of rheumatoid arthritis, and in particular of erosive rheumatoid arthritis.

10. The method of any of claims 1 to 7 wherein a level of more than 31 ng of tenascin-C per ml of serum is diagnostic of rheumatoid arthritis, and in particular of erosive rheumatoid arthritis.

11. The method of claim 3 wherein an increase in the level of tenascin-C in a sample from a subject compared to the level in a normal subject, indicates that it is not appropriate to use an anti-TNF drug.

12. The method of claim 3 wherein an increase in the level of tenascin-C in a sample from a subject compared to the level in a normal subject, indicates that it is appropriate to use an anti-IL17 therapy or another none anti-TNF therapy.

13. The method of claim 11 or 12 wherein the level of tenascin-C in a sample is at least 31 or 33ng/ml of serum.

14. The method of any preceding claim which is carried out *in vitro*.

15. The method of any preceding claim wherein the subject is a mammal.

16. The method of claim 15 wherein the mammal is a human.

17. A kit for use in determining the erosive rheumatoid arthritis status of a subject comprising at least one agent for determining the level of tenascin-C in a sample provided by the subject.

18. The kit of claim 17 wherein the agent is an antibody.

19. The kit of any of claims 17 or 18 further comprising instructions for suitable operational parameters in the form of a label or separate insert.

20. The kit of any of claims 17 to 19 further comprising one or more tenascin-C samples to be used as standard(s) for calibration and comparison.

21. Use of tenascin-C as a serum biomarker for erosive rheumatic arthritis.

22. Use of the determination of the level of tenascin-C in a blood or serum sample as a means of assessing the erosive rheumatic arthritis status in an individual.

23. Use of the determination of the level of tenascin-C in a blood or serum sample as a means of assessing the rheumatoid arthritis status, and in particular the erosive rheumatoid arthritis status, in an individual.

24. A method of treating an inflammatory condition such as, rheumatoid arthritis and in particular erosive rheumatoid arthritis, in a subject comprising determining the level of tenascin-C in a sample from the subject and administering an inflammatory treatment, and in particular a treatment for rheumatoid arthritis or erosive rheumatoid arthritis based on the level of tenascin-C observed.

25. A method of treating rheumatoid arthritis, and in particular erosive rheumatoid arthritis, comprising determining the level of tenascin-C in a sample from the subject and administering an anti-TNF therapy if the tenascin-C level is greater than the level in a reference sample but less than 33ng/ml, preferably less than 31ng/ml.

26. A method of treating rheumatoid arthritis, and in particular erosive rheumatoid arthritis, comprising determining the level of tenascin-C in a sample from the subject and administering a therapy which is not an anti-TNF therapy if the tenascin-C level is greater than 31ng/ml, preferably greater than 33ng/ml.

27. A method substantially as herein described with reference to the examples and figures.

28. A use substantially as herein described with reference to the examples and figures.

29. A use substantially as herein described with reference to the examples and figures.

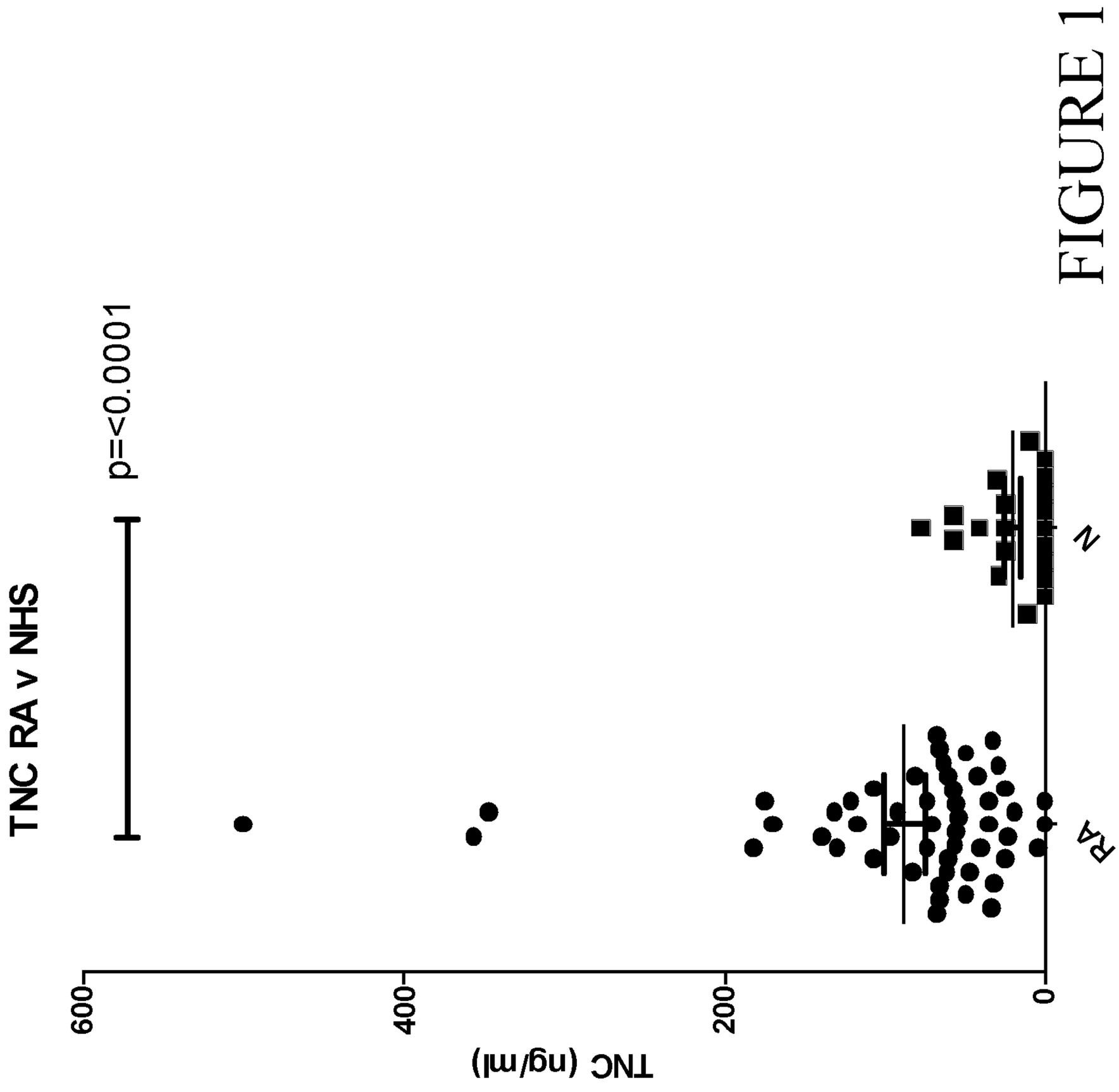


FIGURE 1

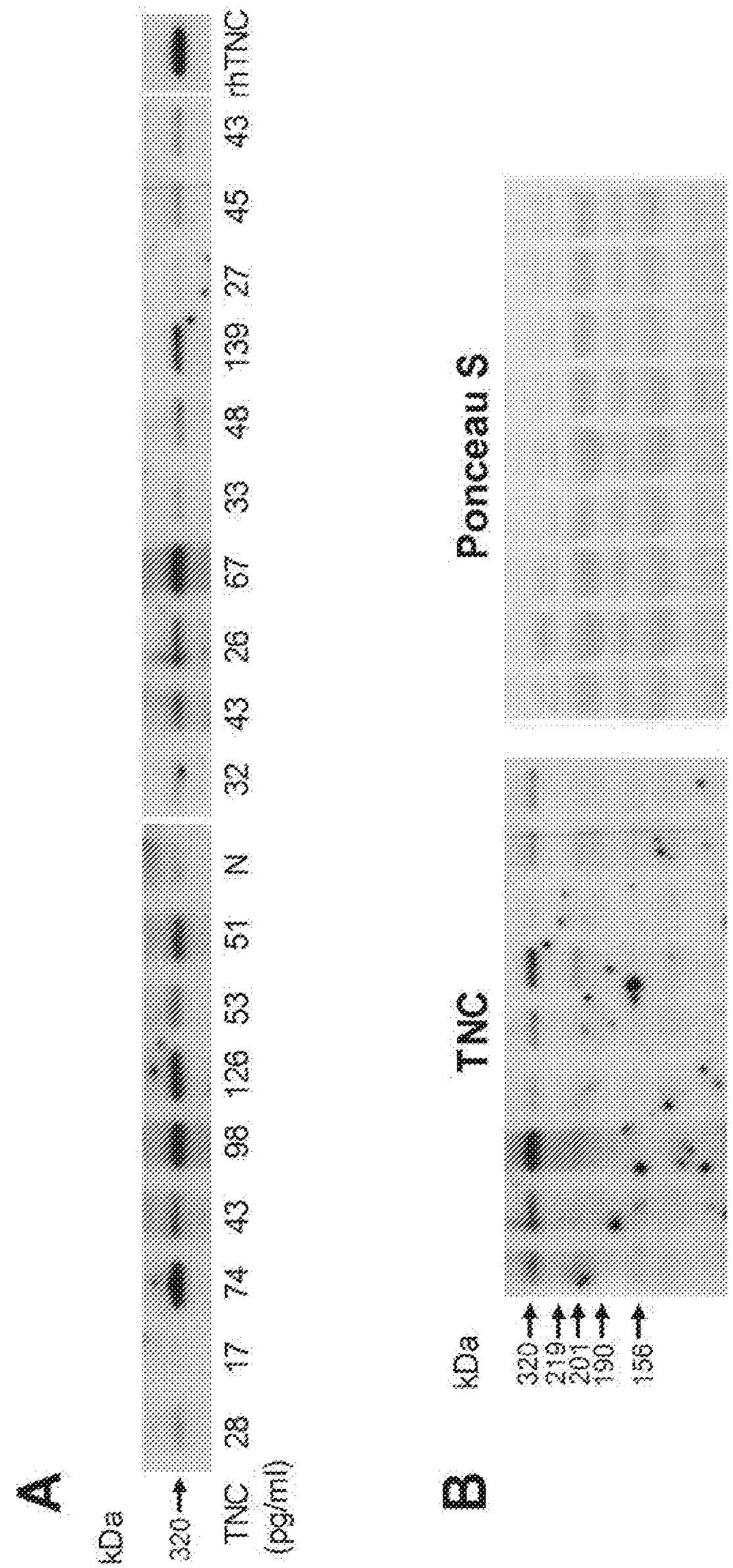

A
kDa
320
TNC
28
(pg/ml)
B
TNC
kDa
320
219
201
190
156
Ponceau S
43
26
67
33
48
139
27
45
43
17
74
43
98
126
53
51
N
32

FIGURE 2

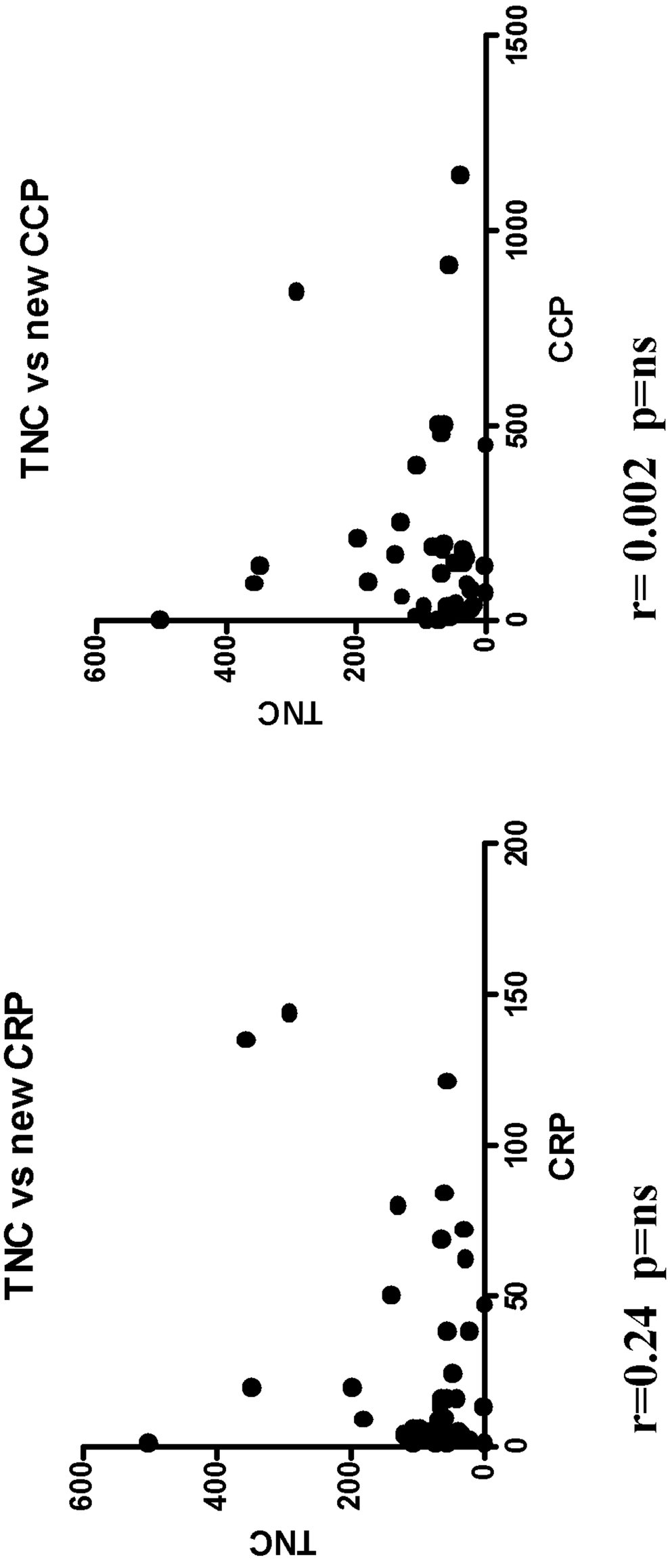

TNC vs new CCP
TNC
600
400
200
0
0
500
1000
1500
CCP
r= 0.002 p=ns
TNC vs new CRP
TNC
600
400
200
0
0
50
100
150
200
CRP
r=0.24 p=ns

FIGURE 3

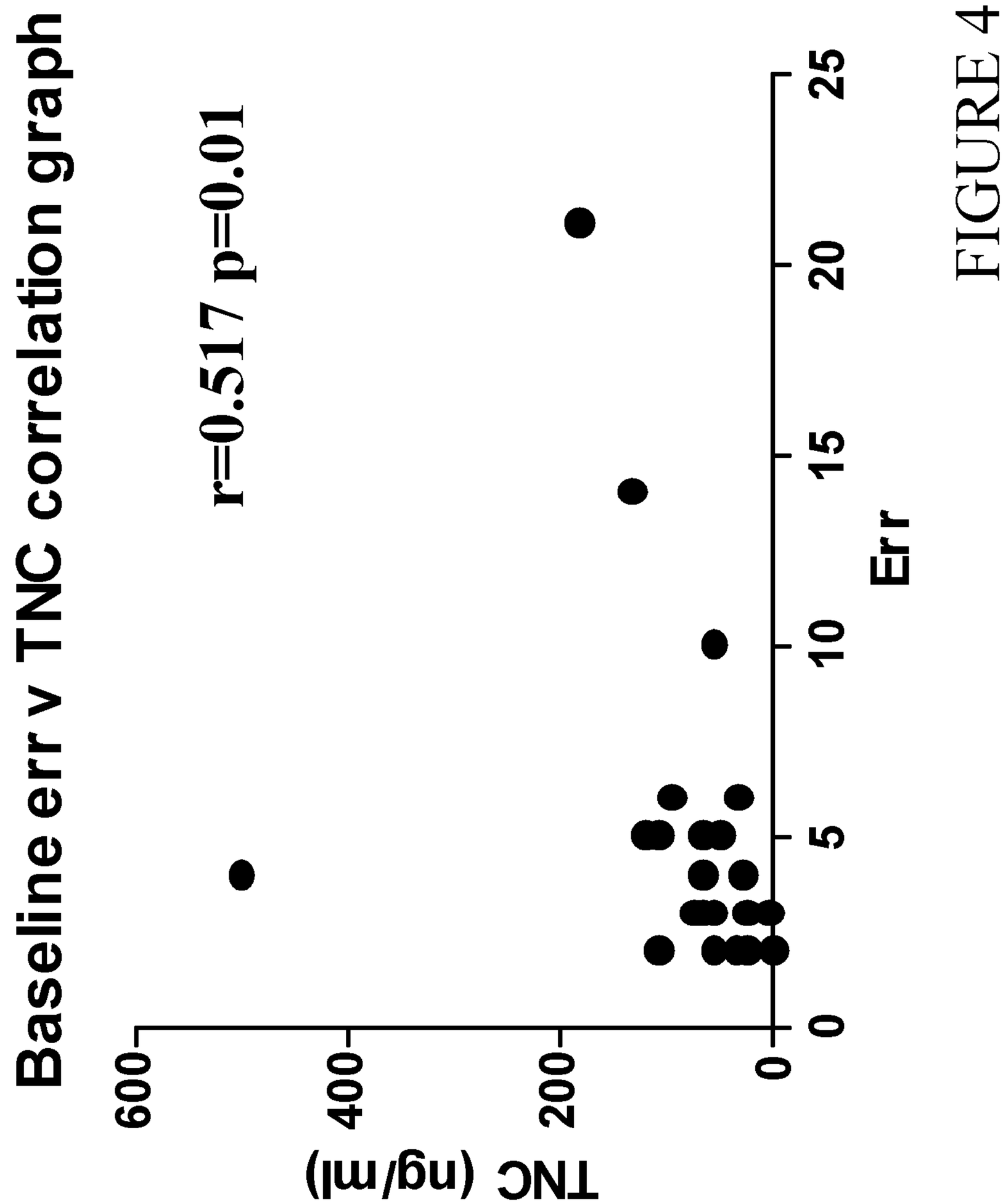

Baseline err v TNC correlation graph
r=0.517 p=0.01
Err rr
0
5
10
15
20
25
0
200
400
600
TNC (ng/ml)

FIGURE 4

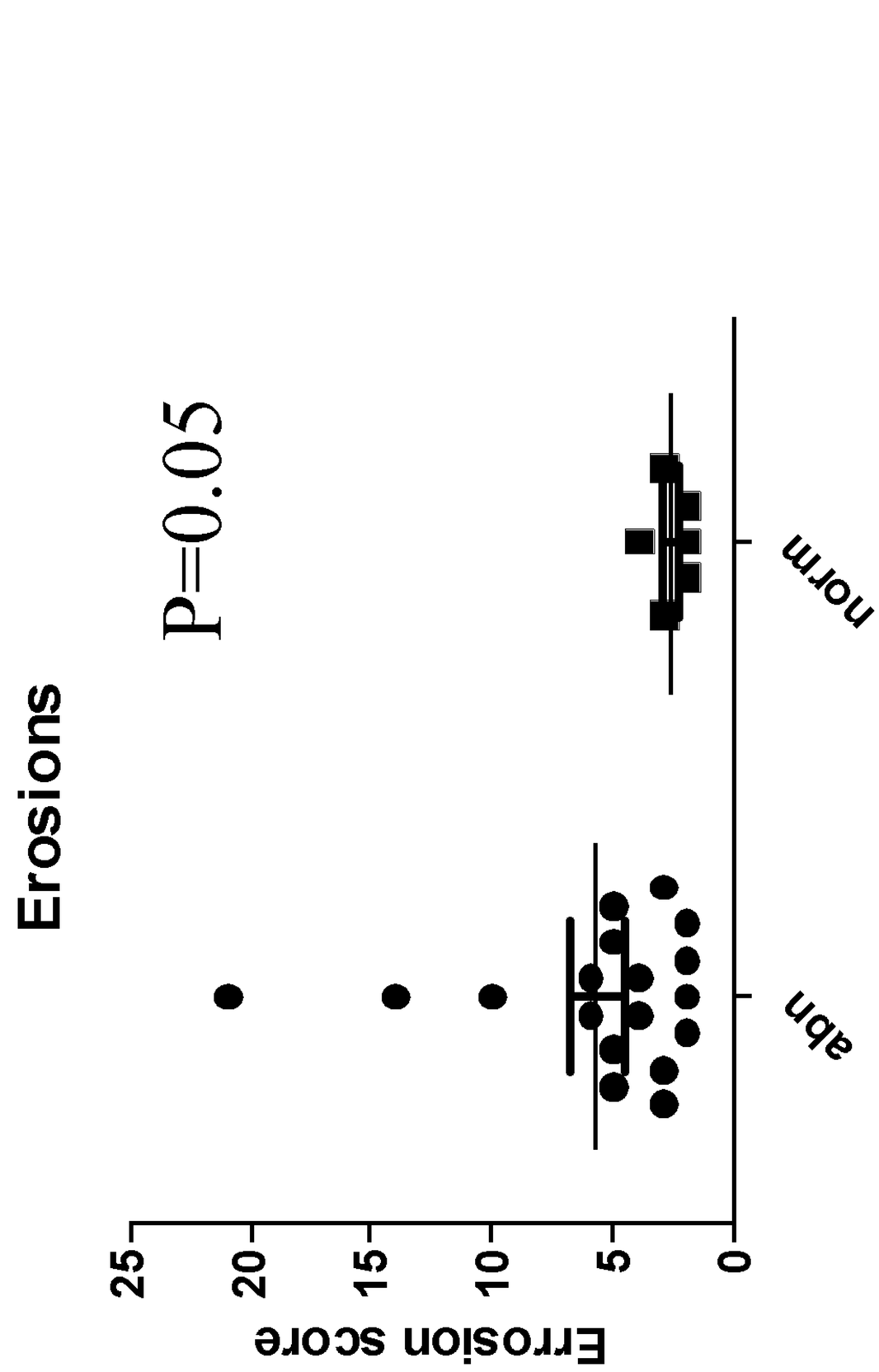


FIGURE 5

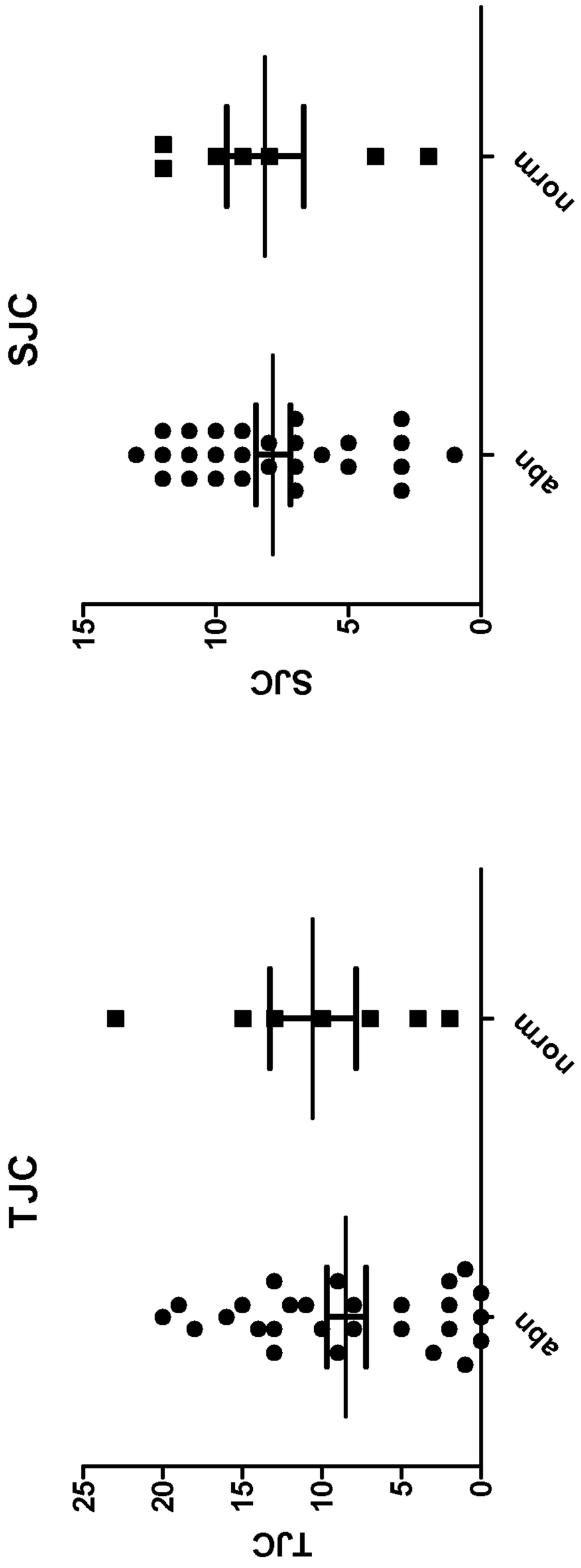
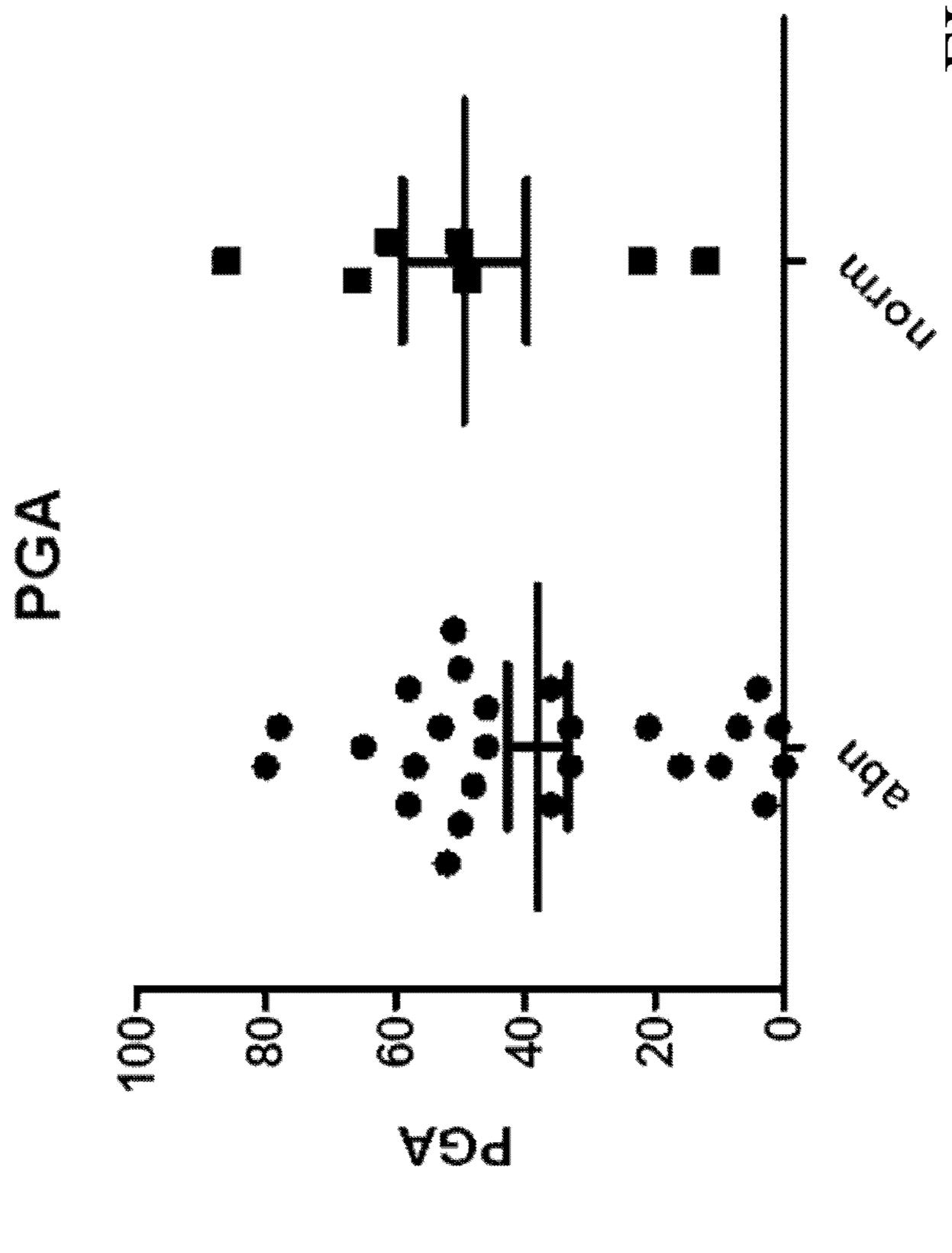


FIGURE 6A

FIGURE 6B



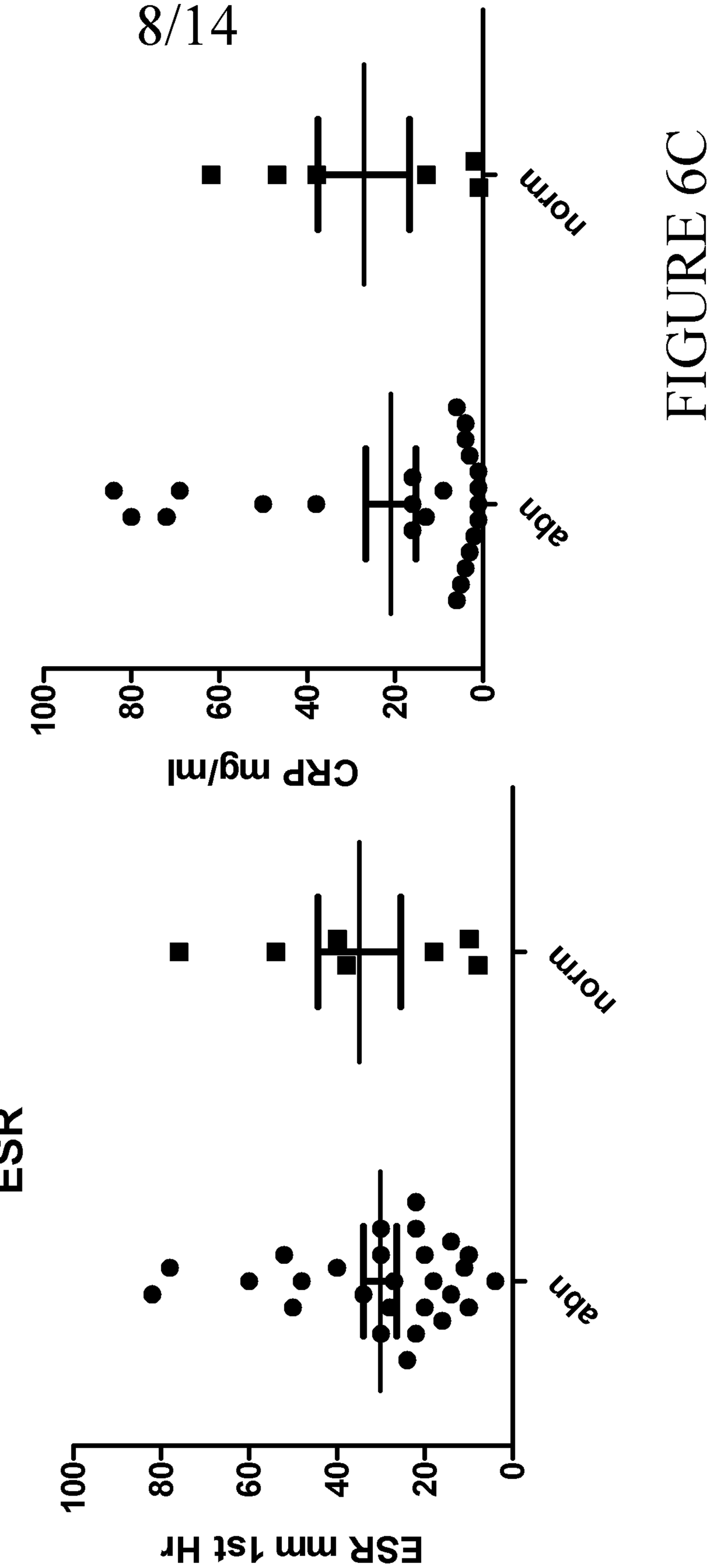

CRP
100
80
60
40
20
0
CRP mg/ml
norm
abn
ESR
100
80
60
40
20
0
ESR mm 1st Hr
norm
abn

FIGURE 6C

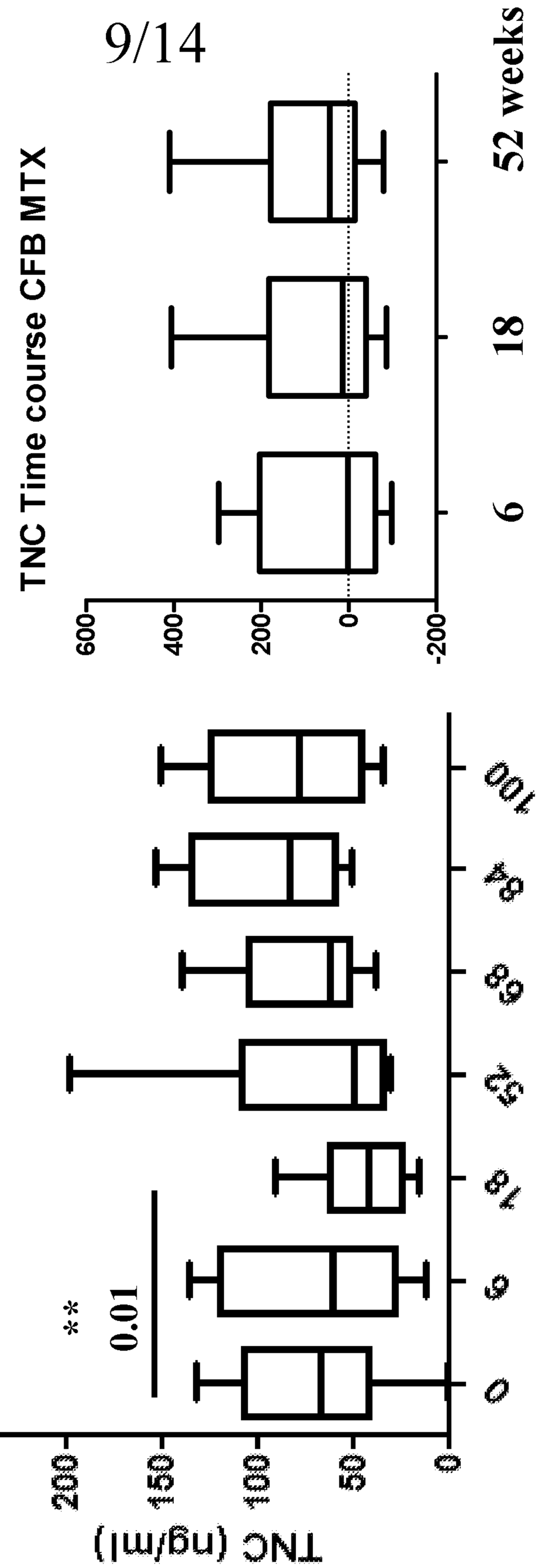

TNC Time course CFB MTX
600
400
200
0
-200
6
18
52 weeks

FIGURE 7

antiTNF+MTX
TNC time course
week
0
4
8
12
16
24
52
100
TNC (ng/ml)
0
50
100
150
200
250
**
0.01

```
        10         20         30         40         50         60
MGAMTQLLAG VFLAFLALAT EGGVLKKVIR HKRQSGVNAT LPEENQPVVF NHVYNIKLPV

        70         80         90        100        110        120
GSQCSVDLES ASGEKDLAPP SEPSESFQEH TVDGENQIVF THRINIPRRA CGCAAAPDVK

       130        140        150        160        170        180
ELLSRLEELE NLVSSLREQC TAGAGCCLQP ATGRLDTRPF CSGRGNFSTE GCGCVCEPGW

       190        200        210        220        230        240
KGPNCSEPEC PGNCHLRGRC IDGQCICDDG FTGEDCSQLA CPSDCNDQGK CVNGVCICFE

       250        260        270        280        290        300
GYAGADCSRE ICPVPCSEEH GTCVDGLCVC HDGFAGDDCN KPLCLNNCYN RGRCVENECV

       310        320        330        340        350        360
CDEGFTGEDC SELICPNDCF DRGRCINGTC YCEEGFTGED CGKPTCPHAC HTQGRCEEGQ

       370        380        390        400        410        420
CVCDEGFAGV DCSEKRCPAD CHNRGRCVDG RCECDDGFTG ADCGELKCPN GCSGHGRCVN

       430        440        450        460        470        480
GQCVCDEGYT GEDCSQLRCP NDCHSRGRCV EGKCVCEQGF KGYDCSDMSC PNDCHQHGRC

       490        500        510        520        530        540
VNGMCVCDDG YTGEDCRDRQ CPRDCSNRGL CVDGQCVCED GFTGPDCAEL SCPNDCHGQG

       550        560        570        580        590        600
RCVNGQCVCH EGFMGKDCKE QRCPSDCHGQ GRCVDGQCIC HEGFTGLDCG QHSCPSDCNN

       610        620        630        640        650        660
LGQCVSGRCI CNEGYSGEDC SEVSPPKDLV VTEVTEETVN LAWDNEMRVT EYLVVYTPTH

       670        680        690        700        710        720
EGGLEMQFRV PGDQTSTIIQ ELEPGVEYFI RVFAILENKK SIPVSARVAT YLPAPEGLKF
```

Figure 8….

730 740 750 760 770 780
KSIKETSVEV EWDPLDIAFE TWEIIFRNMN KEDEGEITKS LRRPETSYRQ TGLAPGQEYE

790 800 810 820 830 840
ISLHIVKNNT RGPGLKRVTT TRLDAPSQIE VKDVTDTTAL ITWFKPLAEI DGIELTYGIK

850 860 870 880 890 900
DVPGDRTTID LTEDENQYSI GNLKPDTEYE VSLISRRGDM SSNPAKETFT TGLDAPRNLR

910 920 930 940 950 960
RVSQTDNSIT LEWRNGKAAI DSYRIKYAPI SGGDHAEVDV PKSQQATTKT TLTGLRPGTE

970 980 990 1000 1010 1020
YGIGVSAVKE DKESNPATIN AATELDTPKD LQVSETAETS LTLLWKTPLA KFDRYRLNYS

1030 1040 1050 1060 1070 1080
LPTGQWVGVQ LPRNTTSYVL RGLEPGQEYN VLLTAEKGRH KSKPARVKAS TEQAPELENL

1090 1100 1110 1120 1130 1140
TVTEVGWDGL RLNWTAADQA YEHFIIQVQE ANKVEAARNL TVPGSLRAVD IPGLKAATPY

1150 1160 1170 1180 1190 1200
TVSIYGVIQG YRTPVLSAEA STGETPNLGE VVVAEVGWDA LKLNWTAPEG AYEYFFIQVQ

1210 1220 1230 1240 1250 1260
EADTVEAAQN LTVPGGLRST DLPGLKAATH YTITIRGVTQ DFSTTPLSVE VLTEEVPDMG

1270 1280 1290 1300 1310 1320
NLTVTEVSWD ALRLNWTTPD GTYDQFTIQV QEADQVEEAH NLTVPGSLRS MEIPGLRAGT

1330 1340 1350 1360 1370 1380
PYTVTLHGEV RGHSTRPLAV EVVTEDLPQL GDLAVSEVGW DGLRLNWTAA DNAYEHFVIQ

1390 1400 1410 1420 1430 1440
VQEVNKVEAA QNLTLPGSLR AVDIPGLEAA TPYRVSIYGV IRGYRTPVLS AEASTAKEPE

Figure 8….continued

```
      1450       1460       1470       1480       1490       1500
IGNLNVSDIT PESFNLSWMA TDGIFETFTI EIIDSNRLLE TVEYNISGAE RTAHISGLPP

      1510       1520       1530       1540       1550       1560
STDFIVYLSG LAPSIRTKTI SATATTEALP LLENLTISDI NPYGFTVSWM ASENAFDSFL

      1570       1580       1590       1600       1610       1620
VTVVDSGKLL DPQEFTLSGT QRKLELRGLI TGIGYEVMVS GFTQGHQTKP LRAEIVTEAE

      1630       1640       1650       1660       1670       1680
PEVDNLLVSD ATPDGFRLSW TADEGVFDNF VLKIRDTKKQ SEPLEITLLA PERTRDITGL

      1690       1700       1710       1720       1730       1740
REATEYEIEL YGISKGRRSQ TVSAIATTAM GSPKEVIFSD ITENSATVSW RAPTAQVESF

      1750       1760       1770       1780       1790       1800
RITYVPITGG TPSMVTVDGT KTQTRLVKLI PGVEYLVSII AMKGFEESEP VSGSFTTALD

      1810       1820       1830       1840       1850       1860
GPSGLVTANI TDSEALARWQ PAIATVDSYV ISYTGEKVPE ITRTVSGNTV EYALTDLEPA

      1870       1880       1890       1900       1910       1920
TEYTLRIFAE KGPQKSSTIT AKFTTDLDSP RDLTATEVQS ETALLTWRPP RASVTGYLLV

      1930       1940       1950       1960       1970       1980
YESVDGTVKE VIVGPDTTSY SLADLSPSTH YTAKIQALNG PLRSNMIQTI FTTIGLLYPF

      1990       2000       2010       2020       2030       2040
PKDCSQAMLN GDTTSGLYTI YLNGDKAEAL EVFCDMTSDG GGWIVFLRRK NGRENFYQNW

      2050       2060       2070       2080       2090       2100
KAYAAGFGDR REEFWLGLDN LNKITAQGQY ELRVDLRDHG ETAFAVYDKF SVGDAKTRYK

      2110       2120       2130       2140       2150       2160
LKVEGYSGTA GDSMAYHNGR SFSTFDKDTD SAITNCALSY KGAFWYRNCH RVNLMGRYGD
```

Figure 8….continued

```
      2170       2180       2190       2200
NNHSQGVNWF HWKGHEHSIQ FAEMKLRPSN FRNLEGRRKR A
```

Figure 8….continued.

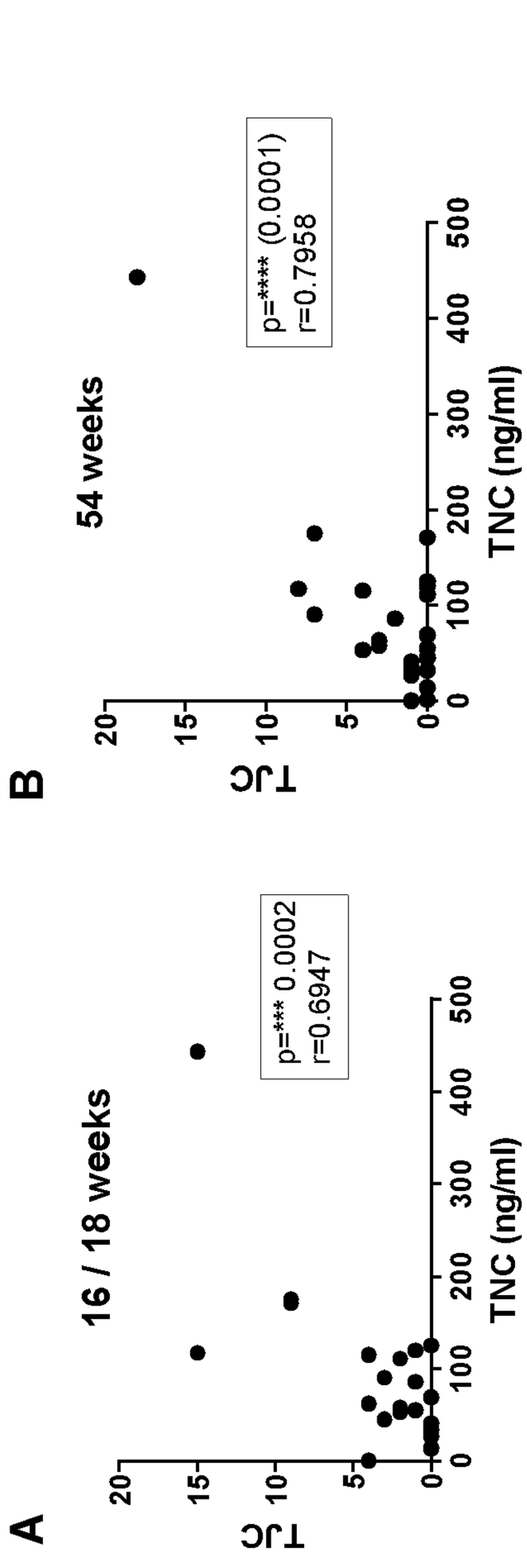

A
TLC
20
15
10
5
0
16 / 18 weeks
p=*** 0.0002
r=0.6947
0
100
200
300
400
500
TNC (ng/ml)
B
TLC
20
15
10
5
0
54 weeks
p=**** (0.0001)
r=0.7958
0
100
200
300
400
500
TNC (ng/ml)

FIGURE 9

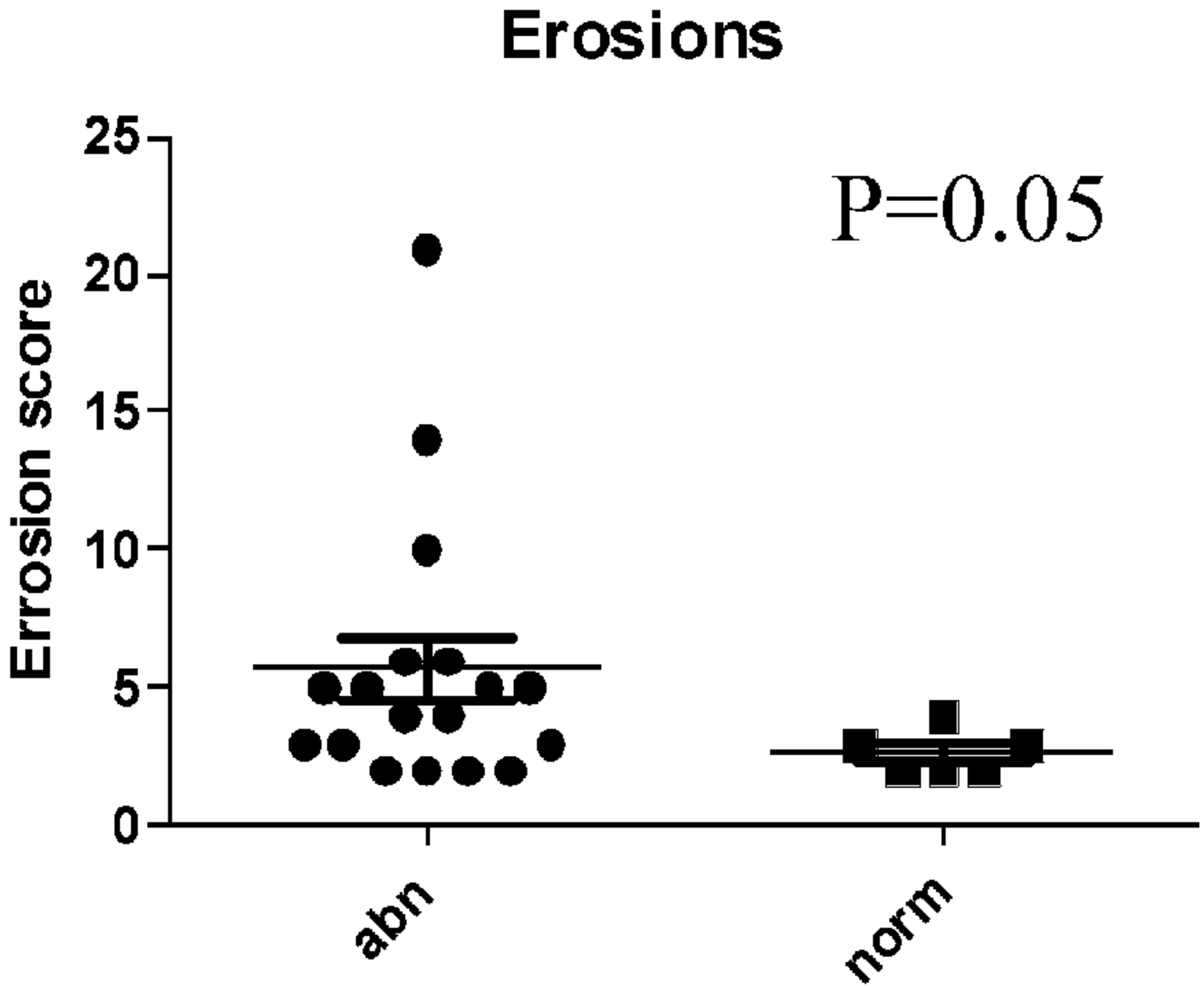


FIGURE 5