

Coagulation indicators and their association with apoptosis ,flammmatory indicators and Sharp scores of rheumatoid arthritis

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Research article

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Abstract

Background

Recent studies have shown that coagulations indicators have a strong correlation with apoptosis, inflammatory indexes. In the present study, we measured coagulation indicators such as PAF, PGI₂ and TXB₂ in patients with RA and investigated their association with apoptosis, inflammatory indicators and Sharp scores of rheumatoid arthritis (RA).

Methods

A total of 90 patients diagnosed with rheumatoid arthritis were enrolled for comparisons. The clinical and laboratory data of patients were retrospectively reviewed. The X-ray findings of both hands were scored according to Sharp's criteria. In addition, the statistics of patients with apoptosis indicator, coagulation indicator and inflammatory indicator. Explored the relationship between coagulation indicators and the above indicators.

Results

In this study, there was a strong correlation between the abnormal increase of apoptosis parameters, immune inflammation indicators, Sharp Score and the increase of coagulation indicator in patients with rheumatoid arthritis, and the increase of Sharp Scores, Caspase-3, FasL indexes or the decrease of C-reactive protein (CRP) could be risk factors for the development of RA. The higher the coagulation indicator, the higher the Sharp score, and the high parameters of hypercoagulable state RA.

Conclusions

Higher coagulation indicators are associated with RA disease activity, as well as apoptosis, inflammatory indicators and decreased physical activity. Moreover, coagulation indicators are associated with Sharp scores. Coagulation indicators are important for the diagnosis of rheumatoid arthritis and are potential markers of activity and hypercoagulable state in RA patients.

Background

Rheumatoid arthritis (RA) is a chronic inflammatory auto-immune disease characterized by persistent synovitis, systemic inflammation, production of autoantibodies, and bone destruction of joints. RA is more frequent among women than men (3:1) and its prevalence is 0.5-1.0% in the adult population^[1, 2]. A large number of clinical studies have shown that coagulation indicators have a strong correlation with apoptosis, inflammatory indexes, PAF, PGI₂ and TXB₂ have been widely confirmed to be associated with immune and apoptosis in RA, PAF is closely related to synovial inflammation and neovascularization

in RA^[3, 4]. Recent studies have found that TxB2/PGI2 pathway can be involved in the pathological changes of angiogenesis of synovial tissue^[5].

Erosive joint damage and bone destruction are the most common manifestation of RA, which might induce ankylosis, malformation, even loss of normal joint function. Current goals of treatment in RA include achieving disease remission, reducing functional disability as well as minimizing pain. Erosions are the hallmark of bone destruction in RA. Radiographic grading of hands is the most commonly used method for the evaluation of different levels of bone erosion in clinical practice. Sharp score have also been used to measure morphological parameters that quantify the bone erosion and bone destruction, giving useful information for early detection and early treatment of RA.

Therefore,we aimed to assess the coagulation index and its associations with apoptosis ,inflammatory indicators and sharp score in RA and to find a common potential cause of the coagulation status in RA patients.

Methods

Patients and study design

A total of 90 RA patients were included in this retrospective study,which were obtained from the First Affiliated hospital of Anhui University of Traditional Chinese Medicine since 2018. All the subjects fulfilled the 2010 ACR/EULAR (American College of Rheumatology/European League Against Rheumatism) criteria for the classification of RA. Sharp score,demographic data,lifestyle factors,clinical parameters and laboratory data were determined by clinical examination or review of electronic medical records. The study was conducted in accordance with the Declaration of Helsinki. Informed consent was obtained from all patients and ethics approval was obtained from the Ethics Committee of the First Affiliated hospital of Anhui University of Traditional Chinese Medicine.

Data Collection and Measures

All RA patients data were recorded soon after hospitalization,which including the details regarding patients'past medical,personal,and family histories. Besides this,RA-related data (age,gender,smoking history,alcohol consumption history,disease duration,treatment-condition) was collected from medical records. On the second day of hospitalization,patients general condition was monitored via vital signs,X-ray radiography of both hands,Sharp scores,blood biochemistry tests,apoptosis index,coagulation index.

X-ray radiography of both hands were performed to assess the extent of joint destruction,bone erosion and joint space narrowing. The Sharp score of each patients was calculated by 2 independent blinded radiologists. Each joint bone erosion was scored as follows: 0 = normal;1 = cell infiltration with no signs of joint erosion; 2 = inflammation with the presence or erosions limited to discrete foci; and 3 = severe and extensive joint erosion with loss of architecture. Each joint stenosis was scored as follows: 0 = normal; 1

= focal or doubtful narrowing; 2 = generalized narrowing < 50%; 3 = generalized narrowing > 50% or subluxation; 4 = complete luxation or bony ankylosis.

Coagulation indexes:human platelet activating factor (PAF),human prostacyclin (PGI2) ,human thromboxane B2 (TXB2)

Flammatory indexes:erythrocyte sedimentation rate (ESR),high-sensitivity C-reactive protein (hs-CRP),rheumatoid factor (RF),anti-citrullinated peptide autoantibodies(anti-CCP),immunoglobulins A(IgA),immunoglobulin G (IgG),immunoglobulin M (IgM),complement 3 (C3),and complement 4 (C4).

Apoptosis indexes:human caspase-3(caspase-3),human caspase-8(caspase-8),human fatty acid synthase (Fas),human factor related apoptosis ligand (FasL),human programmed cell death Protein1 ligand2 (PDL-2),human Bcl2 associated X protein (Bax),human B-cell leukemia/lymphoma 2 (Bcl-2).

Statistical Analysis

Descriptive results were presented as either n (%) or mean (SD). We conducted univariate analyses on important covariates,including age,gender,anti-CCP or RF positivity,PAF,PGI2,TXB2,Sharp score,caspase-3,caspase-8,Fas,FasL,PDL-2,Bax,Bcl-2,ESR,hs-CRP,IGA,IGG,IGM,C3,and C4.Continuous variables with a normal distribution were compared using Student's t-test,while non-normally distributed variables were compared using the Wilcoxon rank sum test. Categorical variables were evaluated using Fisher's exact test. The correlations between Sharp score and coagulation index were analyzed by Spearman's correlation coefficients. In addition,we constructed a binary linear regression model to determine the association of important covariates with Sharp score. Statistical tests were performed in Excel or in GraphPad Prism version 8.0.

The indicators rise was set to "T",while the indicators decline was set to "F". The Aprior module of SPSS Clementine 11.1 software was used to analyze the correlation between Chinese herbal medicines.The most famous association rule is the Apriori algorithm,which aims to find out the relationship between items in a data set,also known as shopping blue analysis. In our data,each drug was treated as a variable.The formulae were as follows:

$$\text{support}(X \rightarrow Y) = \sigma \frac{(X \cup Y)}{N},$$

$$\text{counfidence}(X \rightarrow Y) = \sigma \frac{(X \cup Y)}{\sigma(X)},$$

$$\text{lift}(X \rightarrow Y) = \text{counfidence} \frac{(X \rightarrow Y)}{\sigma(Y)},$$

where $X \rightarrow Y$ is an association rule, X (left-hand side [LHS]) and Y (right-hand side [RHS]) represent the set of herb items, $\sigma(X)$ is the frequency of itemset X , $X \cup Y$ is the union of itemset X and Y , $\sigma(X \cup Y)$ is the frequency with which itemset X and itemset Y appear together, $\text{support}(X \rightarrow Y)$ is the frequency with which X and Y appear together, and $\text{confidence}(X \rightarrow Y)$ is the probability that itemset Y appears in the presence of X . The lift is the ratio of the probability of itemset Y appearing in the presence of X to the frequency of Y . Support and confidence are often used to eliminate meaningless combinations; lift is the validity of the rules.

Results

Characteristics of the Study Population

The study sample was composed of 90 RA patients, with a median age of 55 years (range 30–85) and of whom 90% were female. The median number of Sharp score was 69.00 (range 21–226.5). The median disease duration was 10.0 (range 0.5–40) years (Table 1).

Table 1
 Characteristics of study population (n = 90)

Quantitative Variables	Median	P(25,75)
Age (years)	55.00	50.00,56.00
Disease duration (years)	10.00	5.00,15.25
	Subjects	Percentage
Gender		
Male	9	10.00
Female	81	90.00
Ever smoker	13	14.44
Ever drinker	15	85.56
RF positivity	76	84.44
Anti-CCP positivity	74	82.22
ESR positivity	82	91.11
Marital status		
Currently married	83	92.22
Single,divorced or widowed	7	7.78
Cytokines	M(P25,P75)	
PAF	153.01(126.93,226.52)	
PGI2	8.85(6.23,12.82)	
TXB2	471.48(401.73,576.72)	
Sharp score	69.00 (35.88,100.63)	
Caspase-3	462.31(260.59,616.49)	
Caspase-8	2626.43(2289.17,3177.57)	
Fas	1317.98(1062.60,1567.82)	
FasL	81.61(62.80,97.56)	
PDL-2	888.20(767.28,1068.36)	
Bax	3.92(2.76,5.14)	
Bcl-2	14.28(11.31,16.77)	
IL-4(pg/ml)	25.45(16.09,35.55)	

Quantitative Variables	Median	P(25,75)
IL-10(pg/ml)	29.03	(21.91,39.45)
IL-11(pg/ml)	123.36	(106.99,152.89)
IL-17(pg/ml)	118.47	(96.15,147.07)
TNF- α (pg/ml)	150.99	(119.29,182.39)
ESR (mm/h)	44.00	(28.00,74.75)
hs-CRP (mg/L)	23.55	(6.62,59.48)
RF (U/ml)	81.00	(24.68,196.00)
anti-CCP (U/ml)	49.10	(15.78,194,75)
IGA (g/L)	2.81	(2.18,3.52)
IGG (g/L)	13.62	(11.08,16.80)
IGM (g/L)	1.38	(0.98,1.85)
C3 (g/L)	102.45	(87.50,118.33)
C4 (g/L)	20.45	(17.55,24.33)

Spearman correlation analysis of Coagulation indicators and apoptosis indicators,flammatory indicators ,Sharp score.

To determine whether correlations existed between Coagulation indicators and apoptosis indicators,flammatory indicators ,Sharp score,a Spearman correlation test was performed. Sharp score,TNF- α „disease duration were all positively correlated with PAF,caspase-3,FasL,RF were all positively correlated with PGI₂,IgG were negatively correlated with PGI₂,IL-11,FasL were all positively correlated with TXB₂.

. (I-J) There was a close positive correlation of the TXB₂ with IL-11,FasL.

Table 2
Spearman correlation analysis of coagulation indicators and apoptosis index,flammmatory indicators,Sharp score.

Index		r	p
sharp score	PAF	0.455	< 0.01
TNF-α	PAF	0.257	0.014
disease course	PAF	0.257	0.015
caspase-3	PGI2	0.092	0.004
FasL	PGI2	0.319	0.002
RF	PGI2	0.25	0.018
IgG	PGI2	0.223	0.035
IL-11	TXB2	0.213	0.043
FasL	TXB2	0.255	0.015

Logistic regression analysis of coagulation index and apoptosis indicators,flammmatory indicators ,Sharp score.

Logistic regression analysis of risk factors of coagulation indicators was carried out. Significant differences in coagulation index was found between RA patients with Sharp score ($p = 0.000$),CRP ($p = 0.023$),caspase-3 ($p = 0.039$),FasL($p = 0.003$),indicating that Sharp score,CRP were risk factors for PAF,that caspase-3,FasL were risk factors for PGI2,that Sharp score,FasL were risk factors for TXB2,the higher expression of Sharp score,caspase-3 and FasL,the higher expression of coagulation index,the higher expression of CRP,the lower expression of coagulation index(Table 2).

Table 3
 Logistic regression analysis of coagulation index and apoptosis indicators, inflammatory indicators, Sharp score.

Index	PAF		PGI2		TXB2	
	B	P	B	P	B	P
ages	-1.121	0.16	0.057	0.160	0.349	0.792
disease courses	0.313	0.785	-0.018	0.757	0.756	0.695
sharp score	0.818	0.000**	0.003	0.780	0.662	0.048*
caspase-3	0.033	0.42	0.004	0.039*	0.036	0.603
caspase-8	0.007	0.588	0.000	0.763	-0.021	0.318
Fas	0.014	0.587	-0.002	0.101	0.002	0.967
FasL	0.441	0.203	0.055	0.003*	1.221	0.038*
PDL-2	-0.013	0.765	-0.002	0.370	-0.042	0.576
Bax	-1.275	0.841	0.050	0.878	-8.929	0.403
Bcl-2	1.305	0.493	-0.085	0.383	2.933	0.359
IL-4	0.691	0.431	0.048	0.289	-1.173	0.426
IL-10	0.02	0.971	0.045	0.119	1.493	0.112
IL-11	-0.345	0.355	0.017	0.378	0.993	0.115
IL-17	-0.152	0.61	-0.020	0.189	-0.147	0.768
TNF- α	0.265	0.266	0.000	0.980	-0.515	0.199
ESR	-0.051	0.891	-0.015	0.437	0.455	0.466
CRP	-0.284	0.017*	-0.008	0.188	-0.224	0.256
RF	0.045	0.322	0.004	0.088	0.107	0.167
anti-CCP	-0.026	0.485	0.002	0.266	-0.055	0.369
IgA	12.511	0.121	-0.094	0.819	-1.320	0.922
IgG	-4.08	0.104	-0.117	0.360	-1.713	0.681
IgM	-23.748	0.119	0.347	0.654	-23.966	0.346
C3	0.339	0.371	0.021	0.276	0.189	0.766
C4	1.041	1.702	-0.055	0.529	1.091	0.704

Association rules analysis of coagulation index and apoptosis indicators, inflammatory indicators ,Sharp score.

Association rules analysis of coagulation indexes and apoptosis indicators, inflammatory indicators ,Sharp score can be found in Table 3. Set the minimum support to 80% and the minimum confidence to 80%. Through Aprior module analysis, the correlation between coagulation indexes and apoptosis indicators, inflammatory indicators ,Sharp score was obtained, and the degree of lift was more than 1 and $P \leq 0.05$.

Table 4. Association rules analysis of coagulation index and apoptosis indicators, inflammatory indicators ,Sharp score.

Items(LHS \Rightarrow RHS)	Support	Confidence	Lift	P value
{ PAF \uparrow } \Rightarrow { sharp score \uparrow }	83.68%	92.06%	1.07	< 0.01
{ PAF \uparrow } \Rightarrow { TNF-a \uparrow }	83.05%	91.96%	1.07	< 0.01
{ PGI2 \uparrow } \Rightarrow { caspase-3 \uparrow }	81.25%	91.53%	1.06	< 0.01
{ PGI2 \uparrow } \Rightarrow { FasL \uparrow }	83.05%	91.51%	1.06	< 0.01
{ TXB2 \uparrow } \Rightarrow { sharp score \uparrow }	87.67%	90.79%	1.05	< 0.01
{ TXB2 \uparrow } \Rightarrow { IL-11 \uparrow }	81.25%	89.59%	1.04	< 0.01
{ TXB2 \uparrow } \Rightarrow { FasL \uparrow }	83.68%	89.04%	1.03	< 0.01

Discussion

The association between coagulation indicators and RA has been reported in clinical studies. PAF is a kind of endogenous phospholipid with biological activity. The most effective potent lipid medium found so far, PAF is closely related to synovial inflammation and neovascularization in RA. In a hurry, Bioactive PAF is present in synovial fluid of chronic arthritis^[3]. PAF not only promotes the synthesis and release of interleukin (IL-1), IL-2, IL-6, and tumor necrosis factor (TNF), but also amplifies the inflammatory response and stimulates the proliferation of synovial cells^[4]. This study found that PAF was positively correlated with TNF-A and was associated with many inflammatory responses. Deng et al. found that a variety of PAF subtypes and PAF-like lipids can activate inflammasomes, leading to apoptosis of IL-1 cells and il-18 maturation. To participate in RA immune inflammation and apoptosis imbalance, mediated the formation of bone destruction. Platelet - activating factor (PAF) mediates NLRP3 - NEK7 rather disturbingly named inflammasomes induction independently of PAFR. The correlation analysis of this study showed that Sharp score, TNF-A and course of disease were positively correlated with PAF, and SharpScore was a risk factor for increased PAF. TxA2 mainly from platelets, A strong product generated from physiological

activity of peanut four dilute acid metabolism, produced by prostaglandin H2 through the thromboxane synthase, it is considered one of the specific markers of platelet activation in vivo. TxA2 basically has two active: it is a strong blood small Plate aggregation can promote platelet aggregation to form thrombus; 2 it is can make blood vessels, and bronchial smooth muscle contraction, the vascular tension has a regulatory effect. The unstable in aqueous solution, 30 seconds to hydrolysis into thromboxane B2 (TxB2). Recent studies have found that TxB2 pathway can be involved in the pathological changes of angiogenesis of synovial tissue^[5]. TxA2 and PGI2 are a pair of arachidonic acid derivatives which are mutually resistant and regulated. TxA2 mainly derived from platelets, it has strong vasoconstriction and platelet aggregation. On the contrary, PGI2 mainly dilates blood vessels and inhibits platelet aggregation, which mainly comes from vascular endothelial cells.

This research also found that caspase-3, FasL, RF were all positively correlated with PGI2, IgG were negatively correlated with PGI2, IL-11, FasL were all positively correlated with TxB2. Apoptosis mainly involves FasL pathway, TNF pathway and granulocyte pathway. FasL system. Fas belongs to the tumor necrosis factor receptor (TNFR) superfamily. Activated T cells can overexpress the death receptor Fas ligand (FasL). Fas binds to FasL as a receptor receiving exogenous apoptotic signals, leading to recruitment of FAS-related death determinant (FADD). FADD binds to caspase-8 pre-molecules in DED clusters to activate apoptosis and activate the enzyme Caspase8. Caspase8 directly activates the apoptotic enzyme Caspase-3. At the same time, activation of Caspase8 can enhance the permeability of mitochondrial membrane and lead to the release of cytochrome C. Cytochrome C complex and monomer of Caspase9 combine to form an apoptosis complex, which activates the intrinsic apoptotic pathway^[6]. (2) the TNF. Membrane type TNF - can activate caspase8 by binding to FADD, exerting effects and inducing apoptosis of fine cells. Granzyme pathway. Granzyme can directly crack the caspase-3 precursor (pro-caspase-3) or indirectly lyse pro-caspase-8 to initiate apoptosis.^[7] Caspase is involved in all the major apoptosis pathways, including FAS/FasL, TNF - /TNFR^[8, 9]. The apoptosis signal transduction initiated by granzyme requires the involvement of Caspase. Caspase-3, 8 and 9 play a major role, and the main mechanism is as follows: Activate THE CASpase-activated DEoxyribonuclease (CAD) and cause DNA fragmentation by inactivating the apoptosis inhibitor ICAD^[10]. Destroy the cell structure, the nuclear fiber layer, destroy the nuclear membrane structure; (3) To disable the function of the regulatory protein, cut the protein modulation RA is a chronic systemic inflammatory autoimmune disease with the synovium as the main target tissue^[11]. The prevalence of RA in China was about 0.32–0.36%, and its pathological features were synovial cell proliferation, inflammatory cell infiltration, synovial pannus formation, and invasion and destruction of cartilage and bone tissue^[12]. Repeated joint inflammation, resulting in the destruction of joint structure, deformity, loss of function^[13]. The etiology of this disease is not yet clear. Currently, there are mainly molecular simulation theory, IgG glycation defect theory, MHCII molecule overexpression, and abnormal apoptosis^[14] to explain the pathogenesis of RA. In recent years, studies have suggested that the apoptosis of RA synovial cells, fibroblasts, lymphocytes, and chondrocytes is abnormal, and synovial hyperplasia is a manifestation of abnormal apoptosis^[15]. The mechanism of APOPTOSIS in RA cells and its correlation with inflammation are mainly involved in FasL pathway, TNF pathway and granulase

pathway.FasL system.Fas belongs to the tumor necrosis factor receptor (TNFR) superfamily^[16]. Activated T cells can overexpress the death receptor Fas ligand (FasL).Fas binds to FasL as a receptor receiving exogenous apoptotic signals,leading to recruitment of FAS-related death determinant (FADD)^[17].FADD binds to caspase-8 pre-molecules in DED clusters to activate apoptosis and activate the enzyme Caspase8.Caspase8 directly activates the apoptotic enzyme Caspase-3^[18].At the same time,activation of Caspase8 can enhance the permeability of mitochondrial membrane and lead to the release of cytochrome C.Cytochrome C complex and monomer of Caspase9 combine to form a apoptosis complex,which activates the intrinsic apoptotic pathway^[19].(2) the TNF.Membrane type TNF - can activate caspase8 by binding to FADD,exerting effects and inducing apoptosis of fine cells.Granulase pathway.Granzyme can directly crack the caspase-3 precursor (pro-caspase-3) or indirectly lyse pro-caspase-8 to initiate apoptosis.Caspase is involved in all the major apoptosis pathways,including FAS/FasL,TNF - /TNFR^[20]. The apoptosis signal transduction initiated by granase requires the involvement of Caspase. Caspase-3,8 and 9 play a major role,and the main mechanism is as follows: Activate THE caspase-activated deoxyribonuclease (CAD) and cause DNA fragmentation by inactivating the apoptosis inhibitor ICAD.Destroy the cell structure,the nuclear fiber layer,destroy the nuclear membrane structure;Make the regulatory protein lose its function,cut the regulatory domain and catalytic domain of the protein,and inactivate the protein^[21].

The association rules in this paper suggest that the increase of parameters of high coagulation state is strongly correlated with the increase of caspase-3,TNF-a,IL-11 and FasL,with the support degree greater than 80%,confidence degree greater than 89%,and improvement degree greater than 1. The Logistic regression analysis of the coagulation parameters and immune indicators showed that the coagulation index was strongly associated with the activity of RA and involved in disease progression as a risk factor.The index of coagulation can be used as an important clinical index to predict bone damage and affect bone erosion.

Conclusions

Higher coagulation indicators are associated with RA disease activity, as well as apoptosis ,inflammatory indicators and decreased physical activity. Moreover, coagulation indicator are associated with Sharp scores.Coagulation indicator are important for the diagnosis of rheumatoid arthritis and are potential markers of activity and hypercoagulable state in RA patients.

Abbreviations

abbreviation	Full name
Bax	Bcl2 Associated X Protein
Bcl-2	B-CellLeukemia/Lymphoma 2
CAD	Caspase-activated deoxyribonuclease
Caspase-3	Caspase-3
CK	Cytokine
CRP	C-reactive protein
C3	Complement3
C4	Complement3
ESR	Erythrocyte sedimentation rate
Fas	Fatty Acid Synthase
FasL	Human factor related apoptosis ligand
hs-CRP	Hypersensitive C-reactive protein
ICAM-1	Intercellular adhesion molecule-1
ICE	Interleukin-1 β converting enzyme
Ig	Immunoglobulin
IL-2	Interleukin 2
IL-5	Interleukin 5
IL-8	Interleukin 8
IL-11	Interleukin 11
IL-17	Interleukin 17
PAF	Platelet Activating Factor
PD-L2	programmed death-ligand 2
TXB2	Thromboxane B2
PGI2	Prostacyclin
RA	Rheumatoid Arthritis
RF	Rheumatoid factor

Declarations

Ethics approval and consent to participate

The research was approved by the Ethics Committee of the First Affiliated Hospital of Anhui University of Traditional Chinese Medicine (approval number: 2019AH-12) and carried out under the Helsinki Declaration.

Before participating in the study, the patients filled in a written informed consent form. A written informed consent was obtained from all the study participants (or their parent or legal guardian in the case of children under 16).

Consent to publish

The research has obtained the informed consent for the publication from that person (or their parent or legal guardian in the case of children under 18) about the details relating to an individual person.

Availability of data and materials

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation to any qualified researcher.

Competing interests

The authors declare that they have no competing interests.

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Author Contributions

XW, JL and JTW contributed to the study design. XW contributed to data analysis, wrote the first draft, and revised the manuscript. JTW and JW contributed to the questionnaire survey on patients, specimens, and data collection. JL supervised the project and helped revise the manuscript. All authors reviewed and accepted the content of the final manuscript.

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Figures

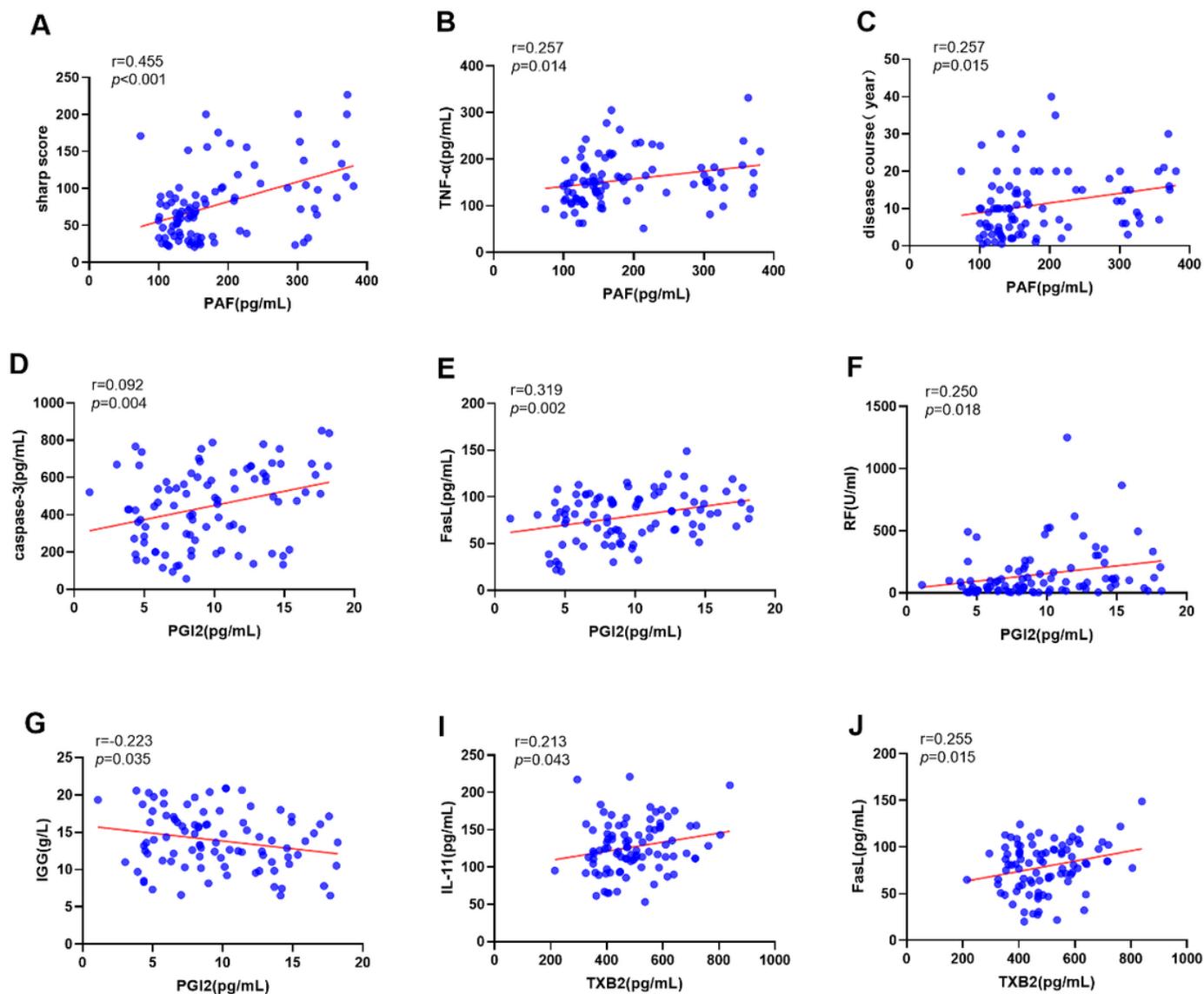


Figure 1

Spearman correlation analysis of coagulation indicators and apoptosis index, inflammatory indicators, Sharp score.

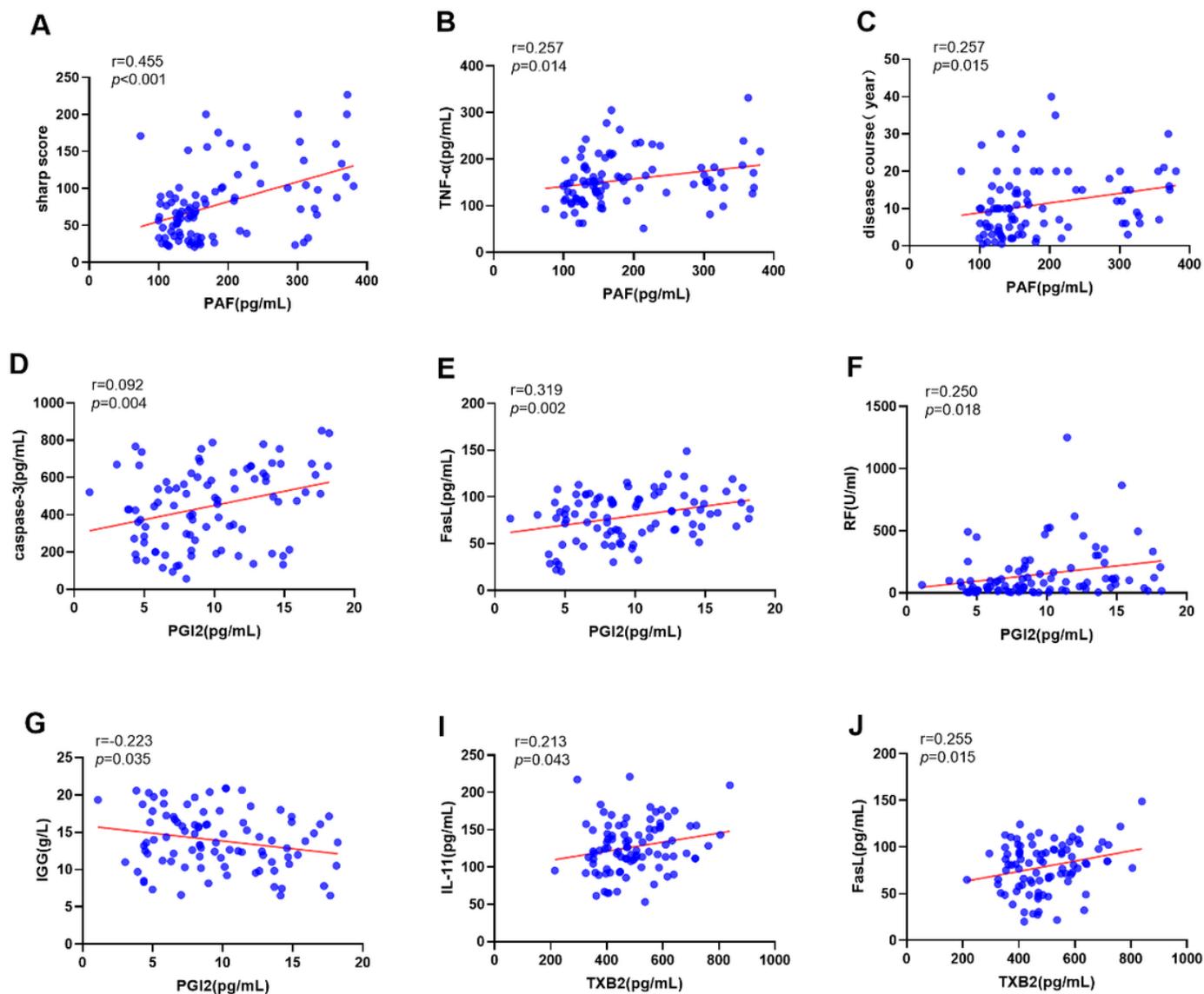


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