A Review on Comparative study of 14-3-3 η protein with conventional serum marker and its role in rheumatoid arthritis.

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Abstract:

Rheumatoid Arthritis in auto immune inflammatory disorder, pathological damage is irreversible in established case of disorder. The 14-3-3 η is a type of inflammatory intracellular protein, which is raised in the case of RA.The main aim of research is to study the prevalence and level of serum 14-3-3 η protein with other convectional Sero makers like ACCP, CRP and RF to identify one of best marker for diagnosis of RA. The Author foundan increase level of 14-3-3 ETA protein compared to ACCP (which is specific marker) CRP, RF level in patients with rheumatoid arthritis. A level of 14-3-3 ETA also increases in case of RF negative arthritic patients. Therefore, the level of serum 14-3-3 ETA protein may be employed to diagnose and categorization of Rheumatic Arthritis patients.

Key Words: **Rheumatoid Arthritis, 14-3-3 eta protein, Arthritis markers.**

Introduction:

RA (Rheumatoid arthritis) is a slowly progressive inflammatory disorder of the system. Which affect nearby 1-2 % population of the world, prevalence ration in female to male is near 3:1. [1]. Main pathology of this disease is joints, swelling which further result in pain, and stiffness with progressive loss of the essential function of that organ or joint. If the patients remain untreated, this may lead to irreversible damage in the joint. In severe cases, as joint damage progresses, it can cause abnormal morphological and biochemical changes (deformities) in the joints that impaired normal function and leads to workplace disability.[1-3]

.One of the most important features of the RD (Rheumatoid disorders) is the inflammation. Generallythe inflammatory response develops after tissue damage to eliminate pathogen. It also limits tissue injury and induced regeneration [2]. All these inflammatory changes are responsible for the increase in some hematological parameter like ESR, some

biochemical parameters like C reactive protein (CRP), some complementary protein and fibrinogen as well there are also decreasing in albumin and some protein level. The serum level of these biomarkers and correlation with clinical condition helps to check diseases progression and therapeutic drug monitoring. However, all these markers are non-specific as they increase in infection and some of malignant disorders. ESR and CRP is the most common test referred by the clinician. Nowadays it is universally accepted that the diagnosis and categorization of RA at early stages and appropriate treatment can help as good prognosis for patients [1-4]

Conventional markers for RA

ACR (American college of rheumatology) and ELAR (European League against Rheumatism) suggest criteria for classification, identification and treatment of rheumatoid disorders. They also suggest good biomarker for diseases [5].Conventional well utilized marker for progression and monitoring rheumatoid arthritis are acute phase reactant like CRP, ESR and antibodies like RF, Anti-CCP or ACPA.

Erythrocyte Sedimentation Rate.

ESR is a simple, easy and cost-effective parameter for evaluating inflammation and acute phase reactions. As the significant action of acute phase protein and cytokines in inflammation is well-established, measurement of ESR is choice of method for evaluating different clinical conditions. Alterations in the inflammatory plasma protein level in circulation like fibrinogen and immunoglobulin which are typically associated with systemic diseases are majority factor that affects ESR level. [7]

By general ESR values is influenced by cellular, Non cellular component, as well as a huge variety of immunological andnon- immunological parameter. It also includesalterations of the morphology and the number of red blood cells, and changes in the normal patterns and level of various plasma proteins.The ESR may rise in a variety of conditions like anemia, pregnancy, tuberculosis,infection, autoimmune disorder and other inflammatory diseases. The Study shows for diagnosis and treatment monitoring of RA patients with only ESR may not very accurately Hence singly ESR not used as an isolated test, but included as part for group of criteria to

diagnose and follow up patients suffering from rheumatoid arthritis and other type of immune arthritis.

C - reactive protein (C.R.P.)

C.R.P.which is one of an acute phase reactant protein generally found in serum following infection or tissue injury caused by many inflammatory processes, trauma or burns. Serum CRP level rises rapidly within 4-6 hrs.[9] and may reached pick level, which is more than 1000 times greater than the reference value. [9]Promoters for synthesis of CRP are Interleukin (IL)-6, interleukin IL-1β, and some of tumor necrosis factorlike (TNF)-α. The magnitude of serum CRP level reflects the level of tissue injury. This will help in monitoring disease's progress.As CRP level is unaltered by age, sex, and morphological abnormality of RBC and different serum proteins. It is comparatively more reliable and objective and useful factor for disease progression to joint damage and further clinical outcome [10,12]

In addition to eliminate infection, dead or damage cell, CRP plays key part in inflammation by activating complement cascade and increasing phagocytosis. Previous study directed us that increase CRP level is not only the results from the inflammation event, but also from pro inflammatory process and bone destruction (by stimulation osteoclastic activity). Which assist in monitoring of tissue, bone damage, clinical presentation in RA

CRP level may be helpful for differentiation of RA from other arthritis like osteoarthritis. However, in very few types of osteoarthritis CRP levels may rise. CRP level is better correlated with radiological progression and treatment response compare to ESR.[10] High level of CRP shows progressive and erosive effects.The high value of CRP also found in tuberculosis, acute infection and other infectious and inflammatory disorders. Slight increase level of CRP found in diabetes, hypertension, depression, alcoholism, smoking, increase activity and chronic tiredness. However, normal or low CRP level doesn't indicate non progressive diseases because about 10% cases of clinically active (RA) shows all acute phase proteins within normal range. [23]

Rheumatoid factor (RF)

RA is one of autoimmune disorder is clinically shown the presence of auto antibodies. RF is a first autoantibodies discovered in rheumatoid arthritis (RA). This develops against Fc part of the immunoglobulin (IgG) which is IgM in nature [15]Majority of RA cases shows RF values greater than 50 U/ml. High RF concentration indicates an aggressive joint disorder, rheumatic or arthritic nodules and further involved in near extra articular tissues. The only RF leveldoesn't diagnose the disease because it may increase in healthy population which also increase with age.

Normally, the temporary production of low binding capacity of IgM F is regularly stimulated by some immunecomplexes and B cell activators. High concentration of RF in synovial fluid of RA stated to increase inflammation and antigen trapping in cavity of joints. [15]

RF (rheumatoid factor) is not just specific for RA, but it may be increased in other rheumatoid disorder like systemic lupus erythematous, infectious arthritis, Sjogren's syndrome, some case of fibrosis and some infectious diseases.[35]30 -50 % early case of RA shows sero-negativity for rheumatoid factor. Although the combinationof serum IgM and IgA antibodytest against RFs isa one of a strong biomarkers of rheumatoid arthritis.But IgA RFs antibodies are not widely and commercially available for validation of test. [14,15]

Anti-Cyclic Citrullinated peptide Antibodies (Anti CCP) and anti-Citrullinated peptide antibodies (ACPA)

Besides the (RF), another group of autoantibodies has been identified in serum of patients with clinical presentation of RA patients are the anti-cyclic citrullinated peptide anti-bodies (anti -CCP).[6]

Previously discovered antibody test isAnti perinuclear factor (A.P.F.) and anti-keratin antibodies (A.K.A). They have a comparatively high specificity of up to 70% positive case found for Rheumatoid arthritis, but it fails to come in routine clinical practice, althoughit has high specificity,. [11]It was because of various technical difficulties in performing the tests. Anti-Sa abs is also found in some type of RApatients. Scientifically it is proved that Anti perinuclear abs, anti-keratin abs, and anti Sa abs. target citrullinated proteins. [15]

Large no of RA patients develops IgG antibodies against citrulline peptides. Previous study determined that the target of these antibodies is one of specific type of protein called filaggrin which may present in synovium of RA patients. When filaggrin is absent in the synovium that time other citrullinated protein like fibronectin, fibrinogen are present in synovium of RA patients. [16]. The Citrullination is a process in which the enzyme peptidyl arginine deiminase (PAD) initiate a posttranslational modification of arginine to citrulline. Naturally, this process occurs in apoptosis, inflammation and keratinization. [1]

The name Anti citrullinated peptide antibody (ACPA) is used after development of non-cyclic citrullinated antibody assays. Like anti- SA antibody assay andanti-mutated citrullinated vimentin assay (anti MCV). Out of them anti MCV has higher sensitivity, but has a lesser sensitivity compare to ACPA for diagnosis of RA. [18]

The ACPAs are the group of autoantibodies, which are found in 70–90% of RA patients. These antibodies rarely present in other disorders and healthy person. Because they have high disease specificity (90–95%). High ACPA level in clinically established patients indicates severe structural damage of joint, radiographic progression diseases and poor therapeutic response. ACPA also strongly associated increased risk with development of RA in healthy individuals before clinical feature develops. [13, 16,17]

14-3-3 eta protein

In 2007, Kilani T. et al first time described the pathophysiological importance of 14,3,3 eta protein in rheumatoid arthritis [42]. The 14, 3, 3 protein is a specific family of intracellulargroup of protein (Chaperone protein), acidic in nature. On the basis of HPLC it shows seven isomers which are divided into α /β, ε, γ, η, ζ, σ, and θ. [25] This 14, 3, 3 protein play important role in proliferation, cell regulation, signal transductions, metabolism and other biological function by interacting with other proteins. [30] In RA this biomarker activates inflammatory and pro inflammatory cofactor. Out of 7 only 14-3-3 eta protein isoform presents in in the serum and joint fluid three to five-fold higher than normal serum level of patients with arthritis [20,26].

Comparative studies between RA, CRP, ESR, with Anti citrullinated protein antibodies

Data of previous study (2010-2019) on 14-3-3 ETA protein, CRP, ACCP (ACPA), ESR and RF and their role in rheumatic arthritis and other rheumatoid disorders were collected and analyzed for possible ideal marker on the basis of specificity, sensitivity and methods from an early and established case of RA.

Many research studies were carried out to establisha marker for RA. Dogan et al (2004) were carried out study in non RA an and RA patients to compare specificity and sensitivity of marker shows that anti-CCP has high sensitivity (69%) and Specificity (99.9 %) but a combination of RF and ACCP has high specificity (100%) in case of RA. Sensitivity of CRP and ESR is good near 88.2%, but it also present in non RA patients in 70.3% which shows less specificity. [14]

The research work of Kim et al (2010) includes 1753 patients show similar result RF and anti CCP has high specificity (85-87%) compared to ESR and CRP [9]. Malini et al (2017) explains Anti CCP as specific marker (96%). [21]

Study	Parameter	No of Patients	R.F. Positive %	Anti-C.C.P. Positive in %	anti-C.C.P. Negative in %	R.F. Negative in %	High CRP in %	High ESR in %	ESR & CRP higher in %	ACCP and RF Positive in %
Dogan et al (2014)	Total No	199	42	48	-	-	56	62.7	56.3	34.2
	RA patients	135	60	70	-	-	72	83.7	88.2	50.4
	Non-RA patients	64	9	5	-	-	22	70.3	70.3	0
	Sensitivity		59	69	-	-	77	84	73	50
	Specificity		92	99.9	-	-	78	30	78	100
Kim et al (2010)										
	No RA patients	1753	68.72	52.2	12.9	47.8	-	-	-	68.8

	Specificity		85	87.1	-	-	-	-	-	78.6
	Sensitivity		52.2	57.6	-	-	-	-	-	70.6
Malini V. et al. (2017)	No of RA patients	572	91.9	75	25	8.1	91.9		-	69.9
	Specificity		82	96	-	-	-	-	-	-
	Sensitivity		67	79	-	-	-	-	-	-

Table 1. Comparative data of R.F, CRP, ESR, with Anti-CCP in RA patients

Kadavath et al in 2014 studies role of 14-3-3 η in rheumatoid arthritis patients, which described combination of ACCP, RF and 14-3-3 η have high positivity (53.9 %) in RA compared to non-RA patients. [22] Similar research work was done by Maksymowych et al in 2014 which express high specific 92.6% in early as well as established patients with RA. [25] Hirata et al (2015) aid that patients 9 patients from 74 patients who were under treatment became negative for 14-3-3 eta protein [33] Gong et al (2017) estimated 14,3,3, η from control (80), early RA (84) and established RA (175) subjects to study false positivity it was only 5% and positive result was about100% in case of established RA[34]. This research is also supported by Stanley J Naides (2015) work express that 14-3-3 ETA protein were present in serongeative case [41].Further research work of Mohmed et al (2018) [29], Lab Corp (2019) and recent work of O. shovan et al (2019) describes similar results.[20,42],

Study	Parameter	14-3-3 η +	RF+	ACCP +	ACCP+ & RF+	14,3,3η + & ACCP+	14,3 ,3 η +& RF +	ACCP+, RF+, 14-3-3 η +
Kadavath et al (2014)	RA patients (91)	24.1	24.1	28.6	46	28.6	24.1	53.9
	Non RA patients (37)	0	32.4	29.7	29.7	27	27	27
Maksymowych et al (2014)	Early RA (99)	64	57	59	72	72	75	78
	Established RA (124)	77	85	79	88	96	94	96
	Sensitivity	63.6	57	59	88	89	88	90
	Specificity	92.6	85	98	84	92	74	78
Hirata et al. (2015)	Non treated RA patients (149)	74	85	65	-	98	95	-
	Treated RA patients (149)	65	-	-	-	-	-	-
Carrier et al (2016)	Early RA (252)	50.6	55.6	47.2	61.5	62.7	63.9	66.7

	Established RA	-	-	-	-	-	-	-
Gong et al (2017)	Control (80)	5	-	-	-	-	-	-
	Early RA (84)	91.75	-	-	-	-	-	-
	Established RA (175)	100	-	-	-	-	-	-
Mohammed et al (2018)	Early RA (57)	82.5	64.9	68.4	68.4	85.9	82.5	87.7
	Established RA (35)	85.7	71.4	85.7	85.7	91.4	85.7	91.4
Lab Corp (2018)	Early RA	64	57	59	72	72	75	78
	Established RA	77	85	79	88	96	94	96
O. Shovman et al (2018)	Early RA (45)	58	67	71	80	80	73	82
	Established RA (51)	43	-	-	-	-	-	-

Table 2. Comparative study positivity of 13-3-3 η with another marker

Summary:

- 14-3-3 ETA protein is the first proteinsignificantly raiseserum and synovial fluid of RA patients, so the level of this prognostic marker indicated progression and out of pathological damage in individual of RA. It is positive in the early stages of RA.
- Although ACCP andRF value is high compared to 14-3-3 ETA protein, but with confirming with Non RA patients 14-3-3 ETA protein is shown less positivity than ACCP positivity that means it has high specificity.
- Specificity of CRP, ESR is less as they are elevated in other inflammatory disorders. Combination of RF, ACCP and 14-4-3-3η increase the sensitivity of test.
- By comparing all Study authors can conclude that Specificity and Sensitivity of the serum 14-3-3 ETA protein is higher than the other it indicates that it isan ideal marker for diagnosis, differentiation and prognosis of Rheumatoid arthritis.

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Future Scope:

- More study is required to differentiate role of 14,3,3 eta protein in RA with other inflammatory disorder.
- There is a current need of development of easy point of care test for novel 14,3, eat protein, so it will be easy available for diagnosis.
- Large group of study is required to avail this test in routine practice for accurate diagnosis and treatment of Rheumatoid arthritis patients.

References:

[1] C. Tanikawa, K. Ueda, H. Nakagawa, N. Yoshida, Y. Nakamura, and K. Matsuda, "Regulation of protein citrullination through p53/PADI4Network in DNA damage response," Cancer Res., vol. 69, no. 22, pp. 8761–8769, 2009.

[2] T. Sokka and others, "Work disability in early rheumatoid arthritis," Clin. Exp. Rheumatol., vol. 21, no. 5; SUPP 31, pp. S71--S74, 2003.

[3] S. M. M. Verstappen et al., "Overview of work disability in rheumatoid arthritis patients as observed in cross-sectional and longitudinal surveys," Arthritis Care Res. (Hoboken)., vol. 51, no. 3, pp. 488–497, 2004.

[4] M. Vermeer et al., "Implementation of a treat-to-target strategy in very early rheumatoid arthritis: Results of the Dutch Rheumatoid Arthritis Monitoring remission induction cohort study," Arthritis Rheum., vol. 63, no. 10, pp. 2865–2872, 2011.

[5] D. Aletaha et al., "2010 Rheumatoid arthritis classification criteria: An American College of Rheumatology/European League Against Rheumatism collaborative initiative," Arthritis Rheum., vol. 62, no. 9, pp. 2569–2581, 2010.

[6] M. L. Burr et al., "Long-term stability of anti-cyclic citrullinated peptide antibody status in patients with early inflammatory polyarthritis," Arthritis Res. Ther., vol. 14, no. 3, p. R109, 2012.

[7] C. Saadeh, "The erythrocyte sedimentation rate: Old and new clinical applications," South. Med. J., vol. 91, no. 3, pp. 220–225, 1998.

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[8] M. F. Bakker et al., “Performance of a multi-biomarker score measuring rheumatoid arthritis disease activity in the CAMERA tight control study,” Ann. Rheum. Dis., vol. 71, no. 10, pp. 1692–1697, 2012.

[9] K. W. Kim, B. M. Kim, H. W. Moon, S. H. Lee, and H. R. Kim, “Role of C-reactive protein in osteoclastogenesis in rheumatoid arthritis,” Arthritis Res. Ther., vol. 17, no. 1, pp. 1–12, 2015.

[10] N. A. Shadick et al., “C-reactive protein in the prediction of rheumatoid arthritis in women,” Arch. Intern. Med., vol. 166, no. 22, pp. 2490–2494, 2006.

[11] A. Kastbom, G. Strandberg, A. Lindroos, and T. Skogh, “Anti-CCP antibody test predicts the disease course during 3 years in early rheumatoid arthritis (the Swedish TIRA project),” Ann. Rheum. Dis., vol. 63, no. 9, pp. 1085–1089, 2004.

[12] F. Atzeni, R. Talotta, I. F. Masala, S. Bongiovanni, and L. Boccassini, “Biomarkers in RA,” no. July, 2017.

[13] D. Mewar et al., “Independent associations of anti-cyclic citrullinated peptide antibodies and rheumatoid factor with radiographic severity of rheumatoid arthritis,” Arthritis Res. Ther., vol. 8, no. 4, pp. 3–7, 2006.

[14] M. Doğan et al., “Comparison of the diagnostic values in rheumatoid arthritis: Anti-CCP antibodies and other serological tests,” Biomed. Res., vol. 25, no. 3, pp. 381–386, 2014.

[15] D. A. Kim and T. Y. Kim, “RF vs. anti-CCP: Competitor or companion?,” Rheumatol. Int., vol. 31, no. 9, pp. 1257–1258, 2011.

[16] E. Taha and S. Moustafa, “Combination of novel and tradition biomarkers to enhance diagnostic sensitivity and specificity for early diagnosis of rheumatoid arthritis and seronegative rheumatoid arthritis,” Zanco J. Med. Sci., vol. 23, no. 2, pp. 247–258, 2019.

[17] R. Aggarwal, K. Liao, R. Nair, S. Ringold, and K. H. Costenbader, “Anti-citrullinated peptide antibody assays and their role in the diagnosis of rheumatoid arthritis,” Arthritis Care Res., vol. 61, no. 11, pp. 1472–1483, 2009.

[18] F. Renger et al., “Immediate determination of ACPA and rheumatoid factor - a novel point of care test for detection of anti-MCV antibodies and rheumatoid factor using a lateral-flow immunoassay,” Arthritis Res. Ther., vol. 12, no. 3, 2010.

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[19] A. H. M. Mohamed, “Serum Level of 14-3-3η (Eta) Protein as a Diagnostic Marker for Rheumatoid Arthritis and Potential Correlation with Disease Activity,” MOJ Orthop. Rheumatol., vol. 7, no. 4, pp. 3–7, 2017.

[20] O. Shovman et al., “The diagnostic value of 14-3-3η protein levels in patients with rheumatoid arthritis,” Best Pract. Res. Clin. Rheumatol., vol. 32, no. 4, pp. 610–617, 2018.

[21] M. et al V., “Studies on the Significance of Anti-CCP Antibodies in Diagnosing Rheumatoid Studies on the Significance of Anti-CCP Antibodies in Diagnosing Rheumatoid Arthritis Cases in Chennai , Tamil Nadu , India,” no. June, 2017.

[22] S. Kadavath et al., “SAT0211 14-3-3 ETA Protein: A Novel Biomarker for the Diagnosis of Rheumatoid Arthritis:,” Ann. Rheum. Dis., vol. 73, no. Suppl 2, pp. 666.1-666, 2014.

[23] M. Birtane, S. Yavuz, and N. Taştekin, “Laboratory evaluation in rheumatic diseases,” World J. Methodol., vol. 7, no. 1, p. 1, 2017.

[24] N. Carrier et al., “Serum levels of 14-3-3η protein supplement C-reactive protein and rheumatoid arthritis-associated antibodies to predict clinical and radiographic outcomes in a prospective cohort of patients with recent-onset inflammatory polyarthritis,” Arthritis Res. Ther., vol. 18, no. 1, pp. 1–14, 2016.

[25] W. P. Maksymowych et al., “Serum 14-3-3η is a novel marker that complements current serological measurements to enhance detection of patients with rheumatoid arthritis,” J. Rheumatol., vol. 41, no. 11, pp. 2104–2113, 2014.

[26] W. P. Maksymowych et al., “14-3-3H Is a Novel Mediator Associated With the Pathogenesis of Rheumatoid Arthritis and Joint Damage,” Arthritis Res. Ther., vol. 16, no. 2, pp. 1–11, 2014.

[27] W. P. Maksymowych et al., “14-3-3$η$ is a novel mediator associated with the pathogenesis of rheumatoid arthritis and joint damage,” Arthritis Res. Ther., vol. 16, no. 2, p. R99, 2014.

[28] W. P. Maksymowych and A. Marotta, “14-3-3 eta, a Novel Mediator Upregulated by TNF alpha, Reflects Clinical Response to Anti-TNF alpha Therapy.,” in ARTHRITIS AND RHEUMATISM, 2011, vol. 63, no. 10, pp. S162--S163.

[29] M. A. Hassan, R. M. Gouda, W. E. Alsayed, and I. A. Kassem, “ASSESSMENT OF 14-3-3 PROTEIN ETA AND ANTI- CYCLIC CITRULLINATED PEPTIDE (ANTI-CCP) IN

JUVENILE IDIOPATHIC ARTHRITIS VERSUS ADULT ONSET RHEUMATOID ARTHRITIS By," vol. 48, no. 3, 2019.

[30] T. Zeng and L. Tan, "14-3-3η protein: A promising biomarker for rheumatoid arthritis," Biomark. Med., vol. 12, no. 8, pp. 917–925, 2018.

[31] S. Mun et al., "Development of a Novel Diagnostic Biomarker Set for Rheumatoid Arthritis Using a Proteomics Approach," Biomed Res. Int., vol. 2018, no. Das 28, 2018.

[32] A. Mc Ardle, B. Flatley, S. R. Pennington, and O. FitzGerald, "Early biomarkers of joint damage in rheumatoid and psoriatic arthritis," Arthritis Res. Ther., vol. 17, no. 1, pp. 1–12, 2015.

[33] S. Hirata, A. Marotta, Y. Gui, K. Hanami, and Y. Tanaka, "Serum 14-3-3η level is associated with severity and clinical outcomes of rheumatoid arthritis, and its pretreatment level is predictive of DAS28 remission with tocilizumab," Arthritis Res. Ther., vol. 17, no. 1, pp. 1–10, 2015.

[34] X. Gong et al., "Elevated serum 14-3-3η protein may be helpful for diagnosis of early rheumatoid arthritis associated with secondary osteoporosis in Chinese population," Clin. Rheumatol., vol. 36, no. 11, pp. 2581–2587, 2017.

[35] B. Fautrel, H. W. Nab, Y. Brault, and G. Gallo, "Identifying patients with rheumatoid arthritis with moderate disease activity at risk of significant radiographic progression despite methotrexate treatment," RMD Open, vol. 1, no. 1, pp. 1–6, 2015.

[36] A. novikov et al, "Scientific Abstracts Diagnostic value of 14-3-3 (eta) in Rheumatoid Arthritis," Innov. Technol. Tech. Cardiothorac. Vasc. Surg., vol. 12, no. 3_suppl, pp. S57–S64, 2017.

[37] Y. W. Song and E. H. Kang, "Autoantibodies in rheumatoid arthritis: Rheumatoid factors and anticitrullinated protein antibodies," Qjm, vol. 103, no. 3, pp. 139–146, 2009.

[38] S. Zhao et al., "The Apoptosis Regulator 14-3-3 η and Its Potential as a Therapeutic Target in Pituitary Oncocytoma," vol. 10, no. November, pp. 1–8, 2019.

[39] M. H. van Beers-Tas, A. Marotta, M. Boers, W. P. Maksymowych, and D. van Schaardenburg, "A prospective cohort study of 14-3-3$η$ in ACPA and/or RF-positive patients with arthralgia," Arthritis Res. Ther., vol. 18, no. 1, p. 76, 2016.

[40] S. J. Naides and A. Marotta, "14-3-3H in 'Seronegative' Rheumatoid Arthritis," J. Rheumatol., vol. 42, no. 10, p. 1995, 2015.

[41] R. T. Kilani et al., “Detection of high levels of 2 specific isoforms of 14-3-3 proteins in synovial fluid from patients with joint inflammation.,” J. Rheumatol., vol. 34, no. 8, pp. 1650–1657, 2007.

[42] L. Corporation, “14.3.3 eta, Rheumatoid Arthritis.” pp. 12–14, 2019.

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