Serum Anti-C1q: A Proposed Role in Lupus Nephritis

A Systematic Review of Diagnostic Test Accuracy

Submitted For Partial Fulfillment of Master Degree in Clinical Pathology

By

Yara El-Sayed Mohammed

MB BCh, Ain Shams University

Under Supervision of

Prof. Nahla Mohamed Zakaria Yousef

Professor of Clinical Pathology

Faculty of Medicine

Ain Shams University

Prof. Ashraf FawzyNabhan

Professor of Obstetrics and Gynecology

Faculty of Medicine

Ain Shams University

Prof. Salwa Ibrahim Bakr

Professor of Clinical Pathology

Faculty of Medicine

Ain Shams University

Faculty of Medicine

Ain Shams University

2016

Acknowledgment

Thanks to **Allah** who guaranteed me the power to accomplish this work.Firstly,it will never be enough to thank my teacher **Prof. Dr. NahlaZakaria**, she gave me endless support, and was always keen on improving medical practice and never says No for new ideas that may help, without her support I wouldn't be able to complete any work.

Secondly, I really owe a lot to my teacher: **Prof. Dr. Ashraf FawzyNabhan,**he was the first to introduce EBM to us as students in our last year in medical school. He taught us that medicine was never just the data that we receive from older doctors - although they were great ones-, but it is our responsibility to find the best working medication, intervention or diagnostic tool for the patients. His words were crystal clear; either to do it the right way or never do it at all.

It is also a pleasure to express my deep gratitude to **Prof. Dr. Salwa Bakr** for her advices, kindness and continuous help to achieve this work.

Special thanks to: Prof. **Dr. Mostafa El Hussieny** (community medicine), **Prof. Dr. Magdy El Sharkawy** (nephrology), **Dr. Ahmed Mostafa** (cardiology), **Dr. MarwaAnas** (dentistry), **Dr. Abdelrahman Ali** (nephrology), **Dr. AyaMagdy** (internship), **Dr. Vivian farid** (internship), for time and effort they paid in helping me during this work.

Introduction

Lupus nephritis (LN) is a frequent and serious complication of Systemic Lupus Erythematosus (SLE) and is associated with considerable morbidity and mortality. The disease course is characterized by unpredictable flares ***(Korbet et al., 2000 and Reyes-Thomas et al., 2011).***

The conventional laboratory markers used in clinical practice such as serum complement levels and double-stranded DNA antibodies are unreliable indicators of lupus nephritis (LN) as they lack both sensitivity and specificity for prediction of active or relapsing LN. Moreover, serum creatinine is also unsatisfactory as a marker of LN because significant renal damage can occur before it rises ***(Hewitt et al., 2004).*** Other laboratory tests such as proteinuria and urinary sediments are also non-specific markers ***(Brunner et al., 2006).***

Renal biopsy remains the gold standard for the evaluation of LN disease activity. However, it is an invasive procedure and serial renal biopsies are not appropriate in clinical practice. Hence, it is very important to identify noninvasive new biomarkers that are able to predict renal flares and/or reflect its activity. These biomarkers could then be followed serially and would enable timely institution of appropriate treatment before the development of significant inflammatory injury in the kidne***y.*** Early treatment may also lead to early and more complete remission and less chronic kidney damage as well as decrease the patient's total exposure to immunosuppressive medications and their toxicities ***(Fiehn et al., 2003 and Houssian et al., 2004)***.

Evidence in human studies has demonstrated the pathogenic role of measuring serum anti-C1q in renal injury in LN ***(Sinico et al., 2009)***.

Aim of the Work

The aim of this work was to measure the accuracy (the sensitivity, specificity, positive and negative likelihood ratios and diagnostic odds ratio) of serum anti-C1q in relation to the gold standard tests for diagnosing LN whether renal biopsy or proteinuria.

Systemic lupus erythematosus and Lupus nephritis

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease, characterized by a wide range of clinical manifestations and target organs affection (phenotypes) with unpredictable flares and remissions that eventually lead to permanent injury. SLE affects mostly women of reproductive age, with up to 20% of the cases beginning in childhood***(Tucker et al.,1995).***

It is a potentially devastating disease, which can involve practically any organ system. Renal involvement, termed lupus nephritis, significantly increases the morbidity and mortality of SLE patients and requires aggressive immunosuppressive therapy, which unfortunately is associated with a plethora of side-effects. The lupus nephritis histology-based classification system currently in use gives the clinician a tool to predict outcomes and to tailor therapy, albeit with moderate to good success at best***(Giannico and Fogo,2013).***

The overall prevalence and incidence of SLE ranges from 1.4 to 21.9% and from 7.4 to 159.4 cases per 100,000 people, respectively ***(Ortega et al., 2010).***

Lupus nephritis (LN) is one of the most serious SLE complications since it is the major predictor of poor prognosis. The incidence and prevalence of LN varies depending on the studied population. The LN cumulative incidence is higher in people of Asian (55%), African (51%), and Hispanic (43%) ancestry compared with Caucasians (14%). Up to 25% of these patients still develop end-stage renal disease (ESRD) 10 years after onset of renal compromise. In terms of outcome, the 5- and

10-year renal survival rates of LN in the 1990s ranged between 83–93% and 74–84%, respectively ***(Mok, 2010).***

In addition, LN develops early in the course of SLE thus becoming a major predictor of poor prognosis ***(Anaya et al., 2011)***. However, in about 5% of the cases, LN may appear several years after the onset of SLE (i.e., delayed LN). The group with delayed LN is positively associated with Sjögren syndrome (SS), lung involvement, and anti-phospholipid syndrome as compared with early LN (i.e., those SLE patients who develop LN during the first 5 years of the disease)***(Varela et al., 2008).***

Predisposing Factors of SLE:

Socio-demographic factors such as sex, race, and ethnicity were found to play an important role in the incidence of the disease, frequency of its manifestations, and therapeutic response ***(Salgado and Herrera-Diaz, 2012).***

1- Environmental triggers:

These factors may not only exacerbate existing lupus conditions but also trigger the initial onset. They include certain medications (such as some antidepressants and antibiotics), extreme stress, and exposure to sunlight, hormones, and infections. These stimuli cause the destruction of cells and expose their DNA, histones, and other proteins, particularly parts of the cell nucleus ***(Wang et al., 2008).***

2- Genetics of SLE:

Research indicates that SLE may have a genetic link. Lupus does run in families, but no single "lupus gene" has yet been identified. Instead, multiple genes appear to influence a person's chance of developing lupus when triggered by environmental factors. The most important genes are located in the human leucocyte antigen (HLA) region; especially Class II (DR, DQ, DP) and class III (C2, C4,) on chromosome 6, where

mutations may occur randomly (*de novo*) or may be inherited. Other genes which contain risk variants for SLE are shown in **Table (1)** ***(Hahn et al., 2008)*****.**

The concordance rate for lupus is 25% among monozygotic twins and approximately 2% among dizygotic twins, these rates indicate that a genetic contribution is important, but it is not sufficient to cause the disease. Many genes that probably contribute to lupus have been identified by means of whole-genome scans from families in which multiple members have lupus ***(Namjou et al., 2007).***

Table (1): The currently known genes and gene regions associated with SLE **(Hahn et al., 2008)**.

Genes involved in human lupus
1.HLA genes
Extended haplotypes predispose to lupus -HLA-B8/DRB1*0301/DQB1*0201/C4AQO(anti Ro) -HLA-DRB1*1501/DQB1*0602(nephritis, low levels of tumor necrosis factor-α) -HLA-DRB1*0101/DQB1*0402 -HLA-A10/B18/C4A4/C4B2/BFS(associated with C2 deficiency) -DR2 -DR3 -DR2/DQw1(anti Ro) -DR3/DQw2(anti Ro plus anti La) -DR2 with DQw6 or DQw7 and others (anti-Sm) -DR4,DR7 with DQw7,DQw8,DQw6, and others(lupus anti coagulant) -Homozygous deficiency of early complement components(C2,C4)
2.Non HLA genes
-Protein tyrosine phosphatase 22(PTPN22) polymorphism(chromosome1). -C1q (chromosome 1). -FcγRIIA receptor allele (chromosome 1). -Promotor polymorphisms of interleukin-10(chromosome1).

Further studies have shown that the genetic basis of LN predisposition exists in two aspects. On one hand, some susceptibility alleles of candidate genes are associated with LN disease severity. On the other hand, there exists a set of kidney-specific genes that are likely to amplify or sensitize patients to autoimmune pathology of LN. Association studies evaluate candidate genes based on their function in the immune system or their aberrant expression in lupus patients. Some important candidate genes associated with LN are summarized in**Table (2)** ***(Morel, 2007)*.**

Table (2): Candidate genes associated with LN ***(Morel, 2007)***

Gene	**Full name**	**Variation**
Kidney-specific targeting		
FCGR3A	Fcγreceptor III-A	V/F158
FCGR3B	Fcγ receptor III-B	Copy number variation (CNV)
ACE	Angiotensin converting enzyme	Alu I/D A–2518G
MCP-1	Monocyte chemo attractant protein-1	M235T
AGT	Angiotensinogen	T–845C
IL-8	Interleukin-8	675 4G4G indel
PAI-1	Plasminogen activator inhibitor-1	Intron 4 repeat
eNOS	Endothelial nitric oxide synthase	A6936G
EPCR	Endothelial protein C receptor	
Amplification of the autoimmune pathology		

CCR5		D32
SPP1		C707T
HLA-DQA	C-C chemokine receptor 5	DQA\0101
HLA-DQB	Osteopontin	DQB\0201
PCD1	DQ alpha	PD1.3G/A
ER	DQ beta	PpXx
MBL2	Programmed cell death 1	Gly54Asp
UG	Estrogen receptor	A38G
IFNG	Mannose binding lectin 2	Allele 114
	Uteroglobin	
	IFNγ	

3- Hormonal Factors in SLE:

The strongest risk factor for development of SLE appears, to be female sex. The female to male sex ratio of 9:1 in SLE is observed during the peak reproductive years, with a gradual decline in the ratio after menopause ***(Petri, 2002).***

Lupus flares are caused by use of oral contraceptives, administration of estrogen, and ovulation induction regimens suggesting that sex hormones modulate the incidence or severity of disease in patients with SLE. Conversely, ovarian failure (and presumably, reduced estrogen concentrations) has been associated with reduced rate of lupus flares ***(Mok et al., 1999).***

On the other hand, androgens tend to be immunosuppressive. Serum levels of dihydroepiandrosterone (DHEA), an intermediate compound in testosterone synthesis, are found to be low nearly in all patients with SLE; this might be mediated by impaired interleukin-2 (IL-2) production in SLE patients ***(Suzuki et al., 1995).*** Experimental studies demonstrated

that lupus can be ameliorated by oophorectomy or treatment with male hormones ***(Schur, 2002).***

A better understanding of hormonal relationships in SLE could lead to novel and improved application of hormonal immunotherapy ***(McMurray et al., 2003).***

4- Abnormalities in Immune tolerance:

Highly auto reactive B lymphocytes and T lymphocytes are deleted, inactivated, or suppressed in healthy individuals by immune tolerance. Mechanisms of tolerance include deletion (B cells and T cells), anergy (B cells and T cells), B-cell receptor (BCR) editing (a change in the light chain of the antibody expressed by an auto reactive B lymphocyte), cytokine shifts (Th1 to Th2 cytokine shifts during T cell development), and induction of regulatory cells (suppressing B cells and T cells). Tolerance steps occur at several points along cell development, beginning with immature or naive cells in the thymus (T cells) or bone marrow (B cells) and extending to peripheral lymphoid organs (B cells and T) ***(Jacobi et al., 2005).***

There are several points in B cell development during which deletion of auto reactive cells can occur. It was found that the use of certain light-chain genes by populations of B cells from patients with lupus indeed differs from the light-chain repertoire in healthy people; this difference could be due to aberrant receptor editing ***(Dorner et al., 2001).***

The intrinsic check points in B cell development are influenced by sex hormones; second signals (especially CD40/CD40L, both of which are over expressed in SLE B cells and T cells); BAF (B-cell activating factor), which is elevated in some SLE patients; and cytokines that are B cell growth factors, such as IL-6 and IL-10 (both are increased in some SLE patients). Ability of this intrinsic B cell tolerance to proceed in an orderly fashion is altered by external factors, and by genetic background and the

tolerogenic or activated states of antigen presenting cells (APCs), including dendritic cells (DCs) ***(Jacobi et al., 2005).***

Pathogenesis of SLE:

The pathogenesis of SLE is complex. It depends on the co-ordinated interactions bridging innate and adaptive effector arms of the immune system **(Fig. 1 & 2*) (Hicks and Bullard, 2006 & Walter et al., 2012).***

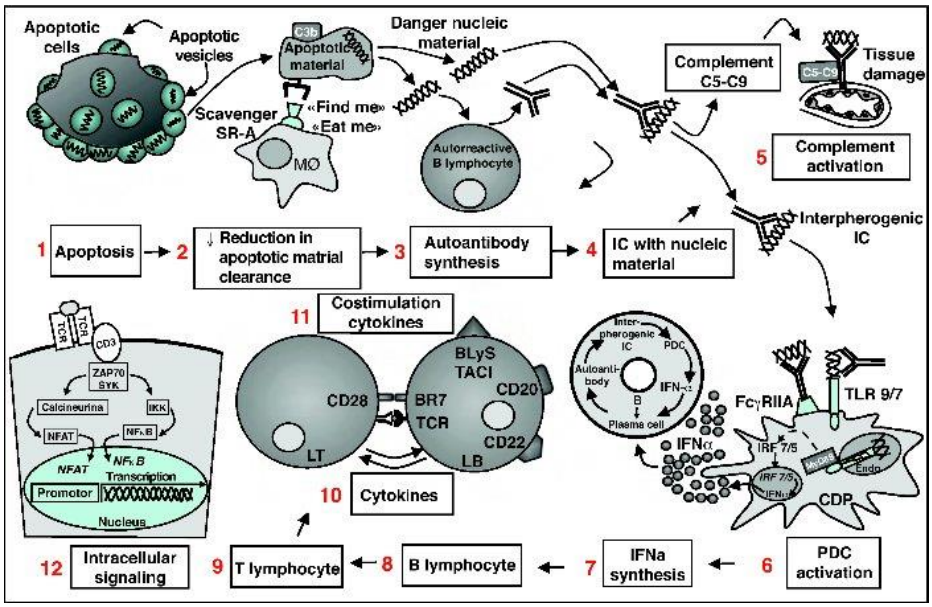

Fig. (1): Panoramic vision of pathogenesis of SLE *(Walter et al., 2012).*

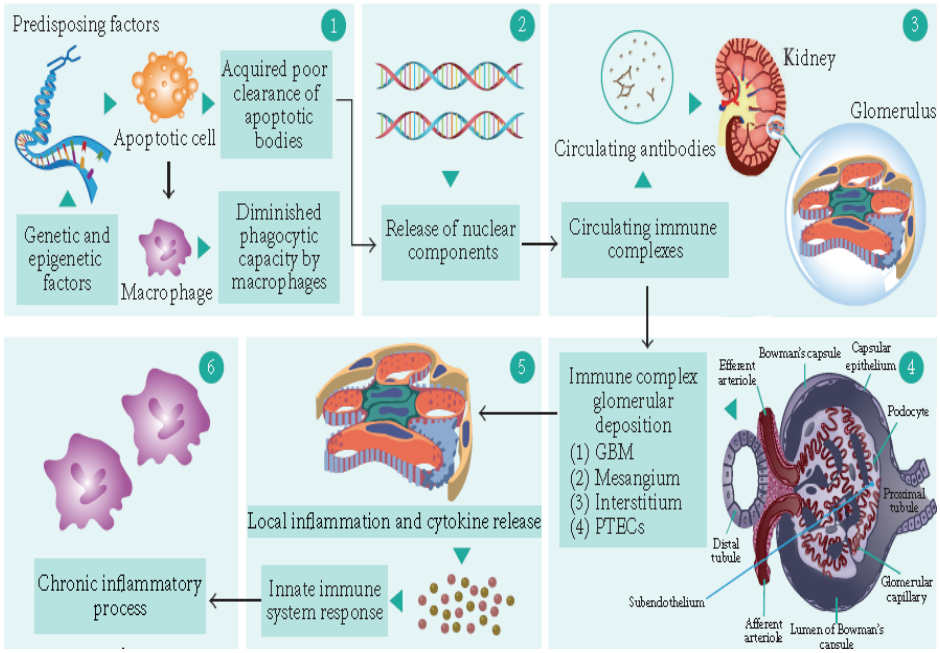

Fig. (2): Lupus nephritis: an imbalance between cytokine homeostasis and IC deposition *(Hicks and Bullard, 2006).*

1- Apoptosis as a source of Auto antigens in SLE:

One manifestation of lupus is abnormalities in apoptosis, (a type of programmed cell death in which aging or damaged cell is neatly disposed of as a part of normal growth or functioning).The obvious source of nucleosomes is the cellular debris released as a result of apoptosis. During apoptosis, blebs of cellular material form on the surface of the dying cell. Antigens that are normally buried within the cells are exposed on the surface of these blebs; and they may trigger an immune response. These exposed antigens include nucleosomes, Ro 62, Ro 50, La, and anionic phospholipids **(Fig. 3)** ***(Rahman et al., 2008)***.

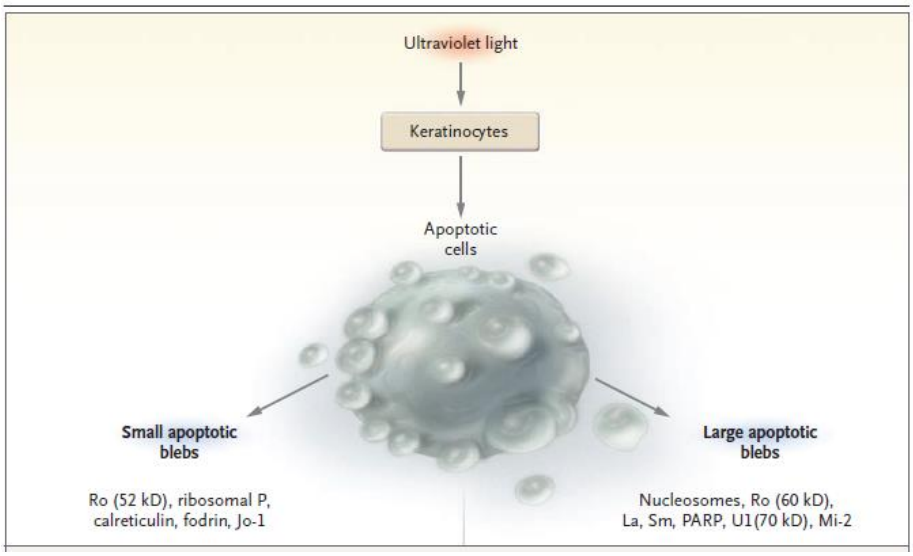

Fig. (3): Induction of Surface Blebs during Apoptosis (PARP denotes poly–ADP–ribose polymerase) ***(Rahman et al., 2008)***.

The clearance of early apoptotic cells is an important function in multicellular organisms. If this ability is disturbed, the apoptosis process progress and finally secondary necrosis of the cells occurs. Necrotic cells release nuclear fragments as potential autoantigens as well as internal danger signals, inducing maturation of DCs, since they have lost their membranes integrity. Increased apoptosis also leads to inefficient clearance that leads to maturation of DCs and also to the presentation of intracellular antigens of late apoptotic or secondary necrotic cells, via MHC molecules. Autoimmunity possibly results by the extended exposure to these nuclear and intracellular autoantigens. In this case B and T cell tolerance for apoptotic cells is abrogated, and the lymphocytes get activated by these autoantigens; inflammation and the production of autoantibodies by plasma cells is then initiated ***(Gaipl et al., 2007).***

Impaired clearance of dying cells is a potential pathway for the development of this systemic autoimmune disease. A clearance deficiency in the skin for apoptotic cells has also been observed in patients with cutaneous lupus erythematosus (CLE). The impaired

clearance may be attributed to deficient phagocytic activity and scant serum components (e.g., complement factors, C-reactive protein (CRP)) in addition to increased apoptosis***(http://wapedia.mobi/en/ Systemic_ lupus_erythematosus.2009).***

In vitro studies proved that the removal of apoptotic debris is abnormal in patients with lupus. Phagocytes from patients with lupus were shown to engulf far less apoptotic material than phagocytes from healthy people during a 7-day culture period ***(Herrmann et al., 1998).***

C1q plays a role in phagocytosis by binding to cell debris, which can then be engulfed by macrophages that have surface C1q receptors. Thus, a deficiency of complement may be an important reason for the poor waste disposal seen in lupus. Homozygous deficiencies of C1q, C2, and C4 are rare disorders, but the presence of any of these genetic conditions was proved to be a strong predisposing factor for lupus ***(Walport, 2002).***

In SLE, monocytes isolated from whole blood of patients show reduced expression of CD44 surface molecules involved in the uptake of apoptotic cells. Most of the monocytes and tangible body macrophages (TBM), which are found in the germinal centres(GC) of lymph nodes and responsible for removal of apoptotic bodies, even show a definitely different morphology in patients with SLE; they are smaller or scarce and die earlier. Serum components like complement factors, CRP -which are decisively important for an efficiently operating phagocytosis- are often missing, diminished, or inefficient **(Fig.4)** ***(http://en.wikipedia.org/wiki/Systemic_lupus_erythematosus#cite_ note18. 2009)***.

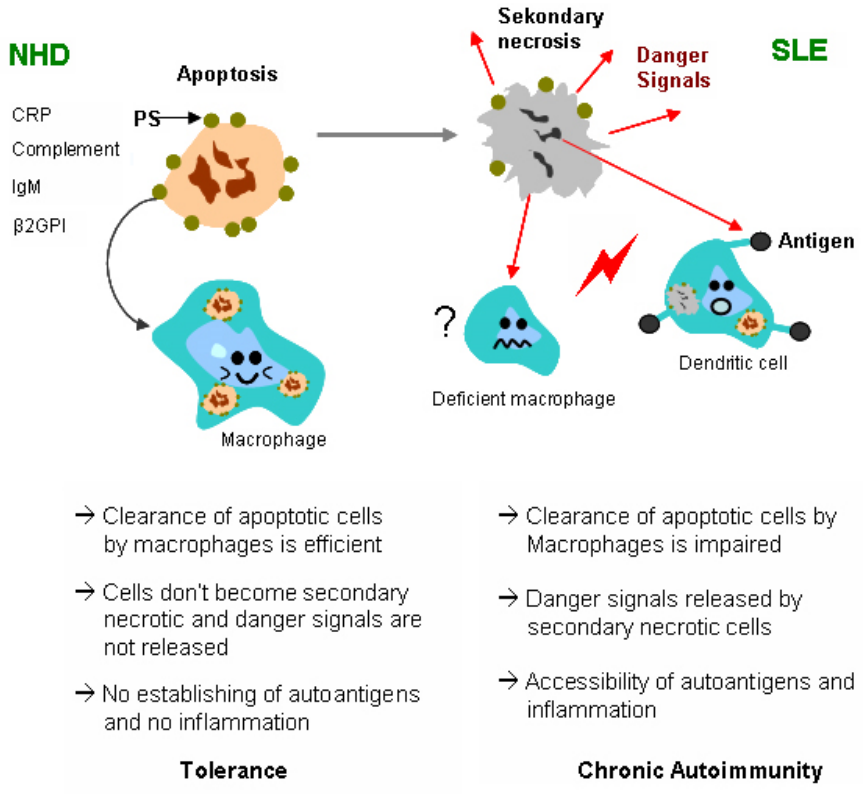

Fig. (4):Defects in the clearance of apoptotic cells*(http://en.wikipedia.org/wiki/Systemic_lupus_erythematosus#cite-note_18.2009)*.

TBMs are large phagocytic cells in the germinal centers of secondary lymph nodes; they express CD68 protein (is a glycoproteinwhich binds to low density lipoprotein. It is expressed on monocytes/macrophages and is used to identify macrophages and giant cells).These cells normally engulf B cells that have undergone apoptosis after somatic hypermutation. In some patients with SLE, significantly fewer TBMs were found, where they rarely contain material from apoptotic B cells. Also, uningested apoptotic nuclei were found outside of TBMs. This material may present a threat to the tolerization of B cells and T cells. Dendritic cells in the GC may endocytose such antigenic material and present it to T cells, activating them. Also, apoptotic chromatin and nuclei may attach to the surfaces of follicular dendritic cells (FDCs) and make