



THE CLINICAL UTILITY OF ANTI-CHROMATIN (ANTI-NUCLEOSOME) IN DIAGNOSING SLE

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Introduction

Anti-chromatin (anti-nucleosome) autoantibodies were one of the first autoantibodies ever detected since they make up the majority of antibodies causing LE Cell formation (Figure 1). Anti-chromatin autoantibodies have had many names over the last few decades: LE cell factor, anti-nucleosome, anti-deoxyribonucleoprotein (DNP), and anti-(H2A-H2B-DNA). These autoantibodies are found in approximately 75% of people with systemic lupus erythematosus (Table 1) and 70% to 100% of people with drug-induced lupus. They are also found in 40-50% of patients with autoimmune hepatitis type 1 (lupoid hepatitis). Anti-chromatin are not generally found in any other disease, thus showing very good sensitivity and specificity for patients with lupus, drug-induced lupus and autoimmune hepatitis type 1. A number of studies have shown that in patients with lupus, anti-chromatin often correlates better with kidney disease than anti-DNA.

Nucleosome-anti-nucleosome immune complexes bind to glomeruli *in vivo*. Anti-chromatin antibodies are ubiquitous in murine models of lupus, and in one strain have been shown to be necessary, but not sufficient, for development of glomerulonephritis. Recent genetic analyses of murine models of lupus have identified at least 3 loci that work together to cause anti-chromatin antibodies and glomerulonephritis in mice. It will be an important breakthrough when the functions of the genes at these loci are identified.

Methodology

Literature Search

A computer search of the scientific literature was performed to find clinical studies that tested the sensitivity and specificity of anti-chromatin (and anti-nucleosome) ELISAs. The results were compiled into Table 1.

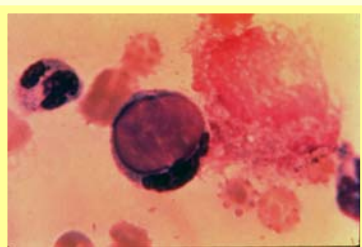
QUANTA Lite™ Chromatin ELISA

Long soluble chromatin from calf thymus was prepared and washed with 0.5 M NaCl to remove histone H1, non-histone proteins and RNA. The resulting antigen is called H1-stripped chromatin and is equivalent to poly-nucleosome core particles (Figure 2). The antigen was coated onto an ELISA plate, and incorporated into an assay that uses the standard INOVA QUANTA Lite™ buffers and incubation times. The results of testing a large number of clinically defined samples from 3 laboratories were compiled into Table 2. Additionally, studies were performed to compare anti-chromatin and anti-DNA (Table 3), measure inter- and intra-assay variation (Table 4), cross reactivity with other autoantigens, and the effect of common interfering substances (not shown).

References

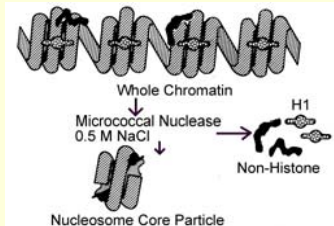
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Figure 1. LE Cell



The LE (Lupus Erythematosus) Cell is formed when anti-chromatin antibodies opsonize the exposed nucleus of a dead cell and the nucleus is engulfed by a granulocyte. The presence of these cells is part of the ACR criteria for lupus.

Fig. 2 The structure of chromatin



The structure of chromatin and the production of nucleosome core particles. In chromatin, the DNA is wrapped around the core histone (H2A-H2B-H3-H4)₂ octamer, and histone H1 and non-histones are bound to the DNA. After treatment with micrococcal nuclease and 0.5 M NaCl the linker DNA is digested and H1 and non-histones are removed, leaving the nucleosome core particle. H1-stripped chromatin is a polymer of nucleosome core particles in which some of the linker DNA is not digested.

Table 1. Sensitivity and clinical correlation of anti-chromatin autoantibody in SLE patients and its specificity in controls with other connective tissue diseases

#SLE patients	Sensitivity	Correlation ^a with kidney disease	#CTD ^b controls	Specificity ^c	Reference
40	78 %	Yes	ND ^d	ND	1
40	48 %	No ^e	ND	ND	2
71	86 %	ND	74	86 %	3
102	46 %	ND	241	99 %	4
120	72 %	Yes, IgG3	376	90 %	5
32	81 %	ND	55	98 %	6
ND	ND	ND	68	100 %	7
136	56 %	Yes	309	97 %	8
129	76 %	Yes	ND	ND	9
100	69 %	Yes	140	92 %	10

^aIn some cases a correlation was mentioned but no statistical test was performed.

^bCTD (Connective Tissue Disease).

^cOnly the controls with connective tissue disease were counted. The group of normal samples was not included.

^dND (not done).

^eA correlation with disease activity was found.

Table 2. Clinical Sensitivity and Specificity for the Chromatin QUANTA Lite™ ELISA.

Clinically Defined	Number	Neg	Pos	Clinical Sensitivity	Reference
SLE	302	106	196	65%	6, 10, 11
DIL	26	6	20	77%	11
AH-1	170	101	69	40%	12
Controls				Clinical Specificity	
Blood Donors	310	308	2	99%	10,11
Scleroderma	79	76	3	96%	6,10
Sjogren's syndrome	100	92	8	92%	10
Cardiac Controls	29	29	0	100%	11
Anti-Phospholipid	30	28	2	93%	10

Table 3. Comparison of anti-chromatin with anti-DNA measured by ELISA or Farr assay in patients with SLE

Number	anti-Chromatin positive	Anti-DNA positive
270	170 (63%)	142 (53%)

Table 4. Precision and Reproducibility

Within-Run

To assess within-run precision of the QUANTA Lite™ Chromatin ELISA, 5 samples were run eighteen times each on one ELISA plate. The results are tabulated below.

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Mean	61 U	49 U	91 U	75 U	133 U
SD	2.9 U	2.7 U	4.1 U	5.2 U	5.1 U
CV	4.8%	5.6%	4.5%	7.0%	3.9%

Between run

To assess between-run precision of the QUANTA Lite™ Chromatin ELISA, 5 positive samples were run once per day on 5 separate days. The results are tabulated below.

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Mean	67 U	57 U	110 U	84 U	167 U
SD	4.3 U	4.1 U	8.6 U	4.7 U	10.2 U
CV	6.4%	7.1%	7.8%	5.6%	6.1%

Conclusions

- The QUANTA Lite™ Chromatin ELISA is a sensitive test for patients with SLE since more than 65% were positive for anti-chromatin antibodies.
- The QUANTA Lite™ Chromatin ELISA is very specific for patients with SLE since only 6% (11 of 179) of patients with Scleroderma or Sjogren's syndrome were positive for anti-chromatin antibodies.
- Anti-chromatin is more sensitive than anti-DNA for patients with SLE, and is more correlated with glomerulonephritis in patients with SLE.
- A high percentage of patients with procainamide-induced lupus and autoimmune hepatitis type 1 (Lupoid hepatitis) are also positive for anti-chromatin antibodies (77% and 40%), respectively.
- The QUANTA Lite™ Chromatin ELISA shows good reproducibility and is cleared by the FDA for *in vitro* diagnostic use.