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What are serum free light chains?

Introduction: At present, patients with symptoms of multiple myeloma or related plasma cell disorders are screened for monoclonal proteins by serum protein electrophoresis (SPEP), urine protein electrophoresis (UPEP) and possibly serum or urine immunofixation electrophoresis (SIFE or UIFE). A new more sensitive assay to detect light chains in the serum is described in detail in this article. A list of plasma cell proliferative disorders that produce monoclonal proteins is found in table 1. The free light chains assay is able to uncover low levels of light chains previously undetected by SPEP, UPEP or IFE. The test is helpful in diagnosis and prognosis of paraproteinemias and differentiating polyclonal from monoclonal disorders. There now is sufficient evidence to routinely measure free light chains in the evaluation of patients with plasma cell dyscrasias.

Plasma Cell Proliferative Disorders

Monoclonal gammopathy of undetermined significance (MGUS)…..….	61%
Multiple Myeloma ……	18%
Primary systemic amyloidosis ……	9%
Lymphoproliferative disorders ……	3%
Smoldering Myeloma ……..…….……	3%
Extramedullary plasmacytoma ……	2%
Macroglobulinemia ……...	2%
Other ……	2%

From Mayo Clinic data base (1960 – 2002)

Table 1 *- From Katzmann, Clinical Laboratory News, June 2006.*

Normal Immunoglobulins: There are five types of immunoglobins produced: IgG, IgA, IgM, IgE and IgD. Each immunoglobulin has a heavy and a light chain (see diagram 1). Each plasma cell produces only one type of heavy chain and only one type of light chain. Normally, twice as many kappa as lambda molecules are produced. Immunoglobulins are produced in response to infection and are made from many different clones of plasma cells (polyclonal). A plasma cell disorder produces only one type of immunoglobulin and is derived from one clone of plasma cells (monoclonal). The light chains produced are exclusively kappa or lambda, depending on the clone. Heavy chains and light chains are produced separately within the plasma cell. For some unknown reason, light chains are produced in excess. The excess light chains enter the blood stream as unbound or free light chains. Normal levels of kappa free light chains are between 3.3 and 19.4 mg/L. Normal levels of lambda light chains are between 0.26 and 1.65 mg/L

Immunoglobulin Structure

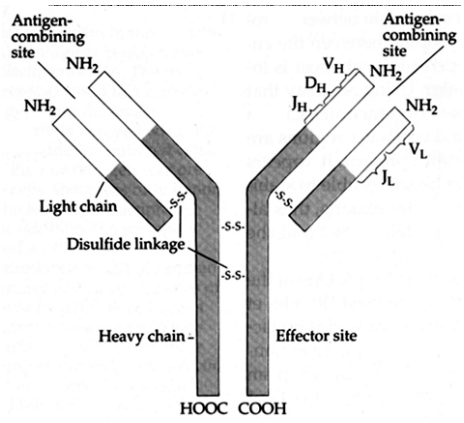

Diagram 1 *- Complete protein (heavy and light chains)*

Measurement of free (unbound) light chains: The development of highly specific in-vitro antibodies permit measurement of unattached free light chains. These antibodies detect specific epitopes that are exposed only when light chains are not bound to heavy chains (see diagram 2). The test is sensitive enough to detect normal levels of free light chains in the serum and small monoclonal amounts that were previously undetected or thought to be "nonsecretory" type of myeloma (see table 2).

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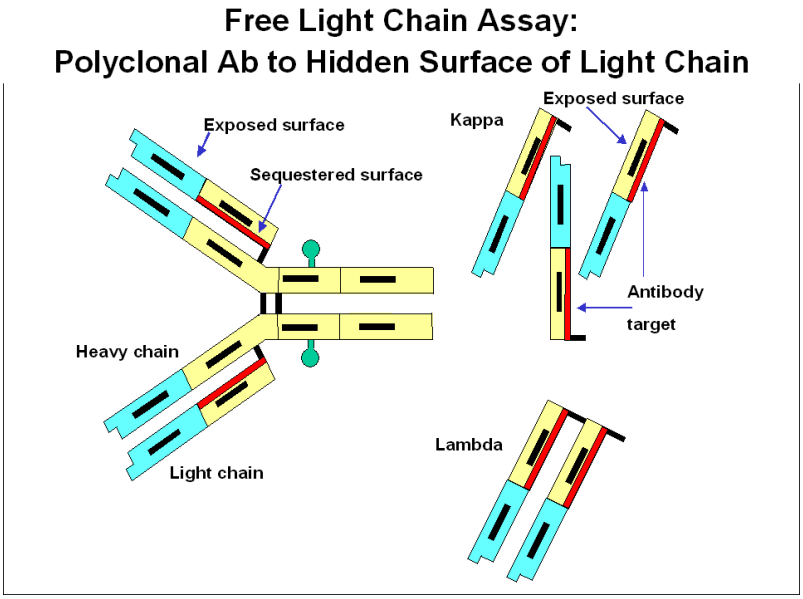

Diagram 2 *- Free Light Chains*

Sensitivity of the Assays

	Kappa	Lambda	Diagnostic finding
	mg/dl	mg/dl	
SPEP	500 to 2000	500 to 2000	monoclonal peak
SIFE	150 to 500	150 to 500	monoclonal peak
FLC	1.5	3.0	abnormal k/l ratio

Table 2 *- Sensitivity levels of three serum assays; SIFE = serum immunofixation, SPEP = serum protein electrophoresis, FLC = Free light chains. (Serum Free Light Chains, 3rd edition, p30.)*

Light Chains and Urine Electrophoresis: With normal renal function free light chains are absorbed and metabolized in the proximal renal tubules. The capacity of the kidney to reabsorb light chains is between 1 and 30 g/24 hours. The normal production of light chains is approximately 500 mg/24 hours. Light chains only appear in the urine when kidney absorption is overwhelmed. Accordingly, urine light chain levels are a reflection of renal tubule function whereas serum levels reflect the production of light chains. The current test to detect excess free light chains in the urine is electrophoresis of concentrated urine. Electrophoresis has replaced the original test (urine for Bence Jones protein) described by Henry Bence Jones in 1847. Unfortunately, detection by electrophoresis requires large amounts of light chains to spill over in the urine before they can be detected. On the other hand, the serum free light chain analysis can detect excess light chains prior to spilling over in the urine.

Kappa/Lambda ratio: The amount of free light chains in the serum may be increased in response to infection (polyclonal production) or renal disease (lack of excretion) as well as a monoclonal proliferative disorder. In renal disease and in normal immune responses, the kappa to lambda ratio remains normal. But in monoclonal proliferations, the kappa to lambda ratio is abnormal because the abnormal plasma cell clone makes only one light chain type. Accordingly, an abnormal k/l ratio reflects a clonal expansion of plasma cells. It is noteworthy, that the serum levels of kappa or lambda light chains are usually elevated in proliferative disorders but they may be within normal limits if the plasma cell clone is small. The normal serum kappa to lambda ratio is 0.26 to 1.65 (see table 3). Levels above or below the normal ratio reflect an imbalance in light chain production. The kappa/lambda ratio is important when distinguishing between monoclonal and polyclonal increases in serum free light chains.

Normal Ranges

	Free light chains	Total light chains
	mg/L	mg/L
Kappa	3.3 to 19.4	2,520
Lambda	5.7 to 26.3	1,430
k/l ratio	0.26 to 1.65	N/A

Table 3 *- Reference ranges for kappa, lambda and k/l ratio. (Mayo Reference Laboratory)*

Polyclonal Gammopathy

Diagnosis	n	Abnormal FLC ratio, %
Normal	282	0
Polyclonal	25	0

Table 4 *- Normal ratio of k/l light chains when polyclonal*

Monoclonal Gammopathy of Undetermined Significance (MGUS): Monoclonal gammopathy of undetermined significance is a premalignant asymptomatic clonal plasma-cell proliferation. In March of this year, Dr. Kyle reported the prevalence of MGUS in the New England Journal of Medicine.[2] He screened 21,000 individuals in Minnesota with serum protein electrophoresis and immunofixation electrophoresis. He found the disorder in 3% of the population 50 years of age or older. There is a greater incidence in men than women and in the elderly. Table 5 shows the prevalence of MGUS according to age and sex:

AGE Years	MEN %	WOMEN %	TOTAL %
50-59	2.0	1.4	1.7
60-69	3.7	2.3	3.0
70-79	5.6	3.8	4.6
>79	8.3	6.0	6.6
Total	3.7	2.9	3.2

Table 5 *- Prevalence of MGUS (Kyle, RA, et. al. NEJM 2006;354:1362-1369)*

By definition, patients with MGUS have a serum M protein of less than 3.0g/dl., bone marrow plasma cells less than 10% and no anemia, hypercalcemia, lytic bone lesions or renal failure. Since MGUS is asymptomatic, the diagnosis is usually the result of a screening serum protein electrophoresis done by a primary care provider. MGUS is associated with progression to myeloma at a rate of 1% per year. Accordingly, lifelong follow-up is necessary in all persons with MGUS.

Serum Free Light Chains Predicts Progression in MGUS: Since myeloma is incurable and has a median survival of only three to four years, delaying or preventing the progression of MGUS assumes great significance. However, chemotherapy is not indicated in asymptomatic MGUS patients. Predicting who will develop multiple myeloma is of utmost importance. Close monitoring could possibly identify who should be studied more extensively or perhaps offered non-toxic treatment prior to expansion of the malignant clone.

It is known that MGUS patients with an abnormal k/l ratio have a greater risk of progression than are those with a normal k/l ratio. Patients with a normal k/l ratio and an IgG M-spike less than 1.5 g/dl have an absolute risk of progression at 20 years of 5% In contrast; patients with an abnormal k/l ratio, a non-IgG protein and M-spike greater than 1.5g/dl have an absolute risk of progression at 20 years of 58% (see Table 6). Clearly monitoring should be conducted more frequently in patients who have all three adverse risk factors.

Progression of MGUS to Myeloma at 20 years

No risk factors……5%
One risk factor……..21%
Two risk factors……..…..37%
Three risk factors……..58%

Table 6 *- Progression of MGUS to myeloma (Rajkumar et. al. Blood;106:812-817)*

Diagnosis of Amyloidosis: Primary systemic amyloidosis is a disorder characterized by the deposition of light chains in organs. It usually presents with heart or renal failure but skin, peripheral nerves and other organs are also frequently involved. Median survival is 12 months from diagnosis although patients who respond well to chemotherapy may live many years. Amyloidosis is a result of a slowly growing clone of plasma cells that secretes light chains, typically lambda. Amyloidosis is one-fifth as common as myeloma.

Amyloidosis is typically difficult to diagnose with the usual methods of serum and urine protein electrophoresis, fat pad aspiration or rectal biopsy. These methods are relatively insensitive unless the light chain concentration is high. Serum and urine immunofixation and serum free light chains are more sensitive in detecting the disease. To establish the diagnosis of amyloidosis, both serum and urine immunofixation and serum free light chains are necessary. These tests are complementary in establishing the diagnosis. Serum free light chains are elevated in over 95% of patients at disease presentation. Finally, the short half-life of light chains in the serum allows for assessing response to chemotherapy in patients with amyloidosis. However, renal failure increases the light chain serum concentration independently of synthesis and can obscure the expected result.

Monitoring Myeloma Treatment: In addition to improved sensitivity, serum free light chain assays offer other advantages for monitoring patient with myeloma. The short half-life of free-light-chains in serum (two - four hours) allows earlier assessment of changes in tumor mass than can be achieved by monitoring changes in intact immunoglobulin (IgG half-life is 20 - 25 days). Monitoring is appropriate in most myeloma patients producing intact immunoglobulin, since 95% also produce excess serum free light chains.

Light Chain and Nonsecretory Myeloma: Among the patients with multiple myeloma, approximately 20% have light chain myeloma. Although these patients have large numbers of plasma cells in the bone marrow, they usually have small or undetectable amounts of monoclonal protein in their serum. The plasma cells in this disorder have lost the ability to make heavy chain immunoglobulins. Studies by Bradwell demonstrate that patients with light chain myeloma have abnormal k/l ratios. [4] Since the body rapidly clears monoclonal light chains from the blood, the residual low concentration may be hidden by the normal quantity of polyclonal light chains. Patients with light chain myeloma are best detected with both urine protein electrophoresis and serum free light chain assay.
In addition, 3% of myeloma patients have a form of the disease call non-secretory myeloma in which monoclonal protein is not detectable in either serum or urine when assessed by protein electrophoresis or immunofixation. By definition, non-secretory myeloma has no detectable serum

or urine monoclonal proteins but has a clonal proliferation of plasma cells. The clonal proliferation can be detected by kappa or lambda immunohistochemical methods on bone marrow biopsies.

Mayo Reference Test: The serum free light chain test is available from the Mayo Clinic Reference Lab at a charge of $127.00. Order as a reference test "immunoglobulin free light chains, serum" (#84190). The results are reported as kappa and lambda free light chains in mg/L and a kappa/lambda ratio with accompanying normal ranges. A red top tube (minimum requirement of 0.5 ml of serum) is sent refrigerated. The CPT code is 83883x2. The test is run Monday through Saturday.

Discussion: Within the last five years a sufficient body of evidence has accumulated to realize that evaluation of serum free light chains is clinically important. Although there are still many unanswered questions, the serum assay adds a level of certainty to our current battery of tests in the diagnosis and management of plasma cell proliferative disorders. As is true with most new laboratory tests, there is initial skepticism, then acceptance (irrational exuberance in some), followed by disillusionment and finally reality.

Stephen V. Chiavetta, MD

References:
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2. *Kyle R et. al., Prevalence of monoclonal gammopathy of undetermined significance, NEJM 354:1362-1369, 2006.*
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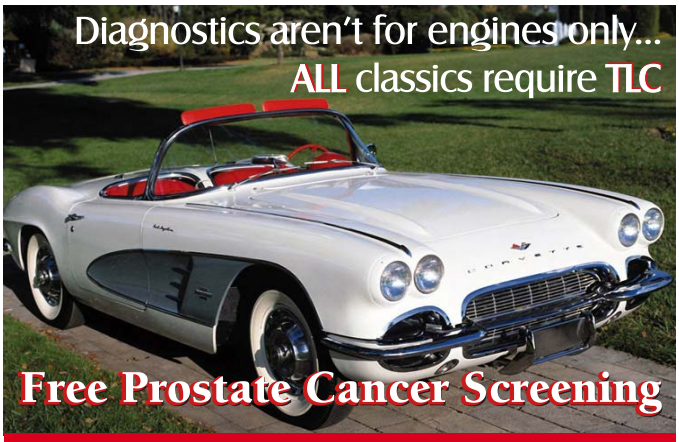

Prostate Cancer Screening
Saturday, September 30, 2006
8am - noon
Rex Senior Health Center
512 E Davie St.
Raleigh, NC

Classic Car Show
Benefiting the Prostate Cancer Assistance Fund
Saturday, September 16, 2006
10 am until 2 pm
Rex Medical Office Building Parking Lot

For more information concerning either of these events, contact **Rex Cancer Outreach (919) 784-2345.**

Laboratory employees are gearing up

Laboratory employees, in cooperation with Rex Cancer Outreach, are gearing upfor the annual Rex Healthcare Prostate Cancer Screening event! This screening is offered free of charge to the public.

Every year, many of the laboratory staff volunteer their time and talents to make this screening a success. The Rex Healthcare Laboratory donates the supplies and manpower needed to assure that each blood specimen is collected in a safe and efficient way and that the PSA testing is performed accurately and in a timely manner.

We are very proud of our laboratory team, many who volunteer at this event every year.