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PARAPROTEIN IDENTIFICATION, MEASUREMENT AND DISEASE ASSOCIATION INFORMATION SHEET

INTRODUCTION

A paraprotein is the immunoglobulin product of a single-cell clone and thus will be of only one heavy chain type (IgG, IgA, IgM or rarely IgD) and/or only one light chain type (kappa or lambda; κ or λ). The paraprotein produced in an individual is composed of immunoglobulin of identical size and charge characteristics and it thus migrates as a discrete band on serum or urine electrophoresis (EPG), which is how they are most commonly identified.

Especially in myeloma, production of immunoglobulin light chains (Bence Jones protein) may markedly exceed that of heavy chains, resulting in an inability to assemble into whole immunoglobulin molecules. In a small proportion of cases of myeloma, the malignant cell clone will produce only light chains and not synthesise heavy chains. This free light chain (Bence Jones protein), is not retained by the glomerular basement membrane and if produced in sufficient excess, passes freely into urine because of its low molecular weight. Because of this, free light chains are not readily detected in serum unless renal impairment is present.

Paraproteins may appear in serum preceding or because of, myeloma or a lymphoproliferative disorder such as a lymphoma or CLL. They may also be an incidental finding (monoclonal gammopathy of undetermined significance, MGUS). The prevalence of MGUS progressively increases from about 1-2% at age 50-60 years to 5-8% in those over 80 [1], and is associated with an increased risk of developing myeloma or lymphoproliferative diseases. In one large populationbased Dutch cohort, the cumulative incidence of MGUS progression to one of these diseases was 0.4% per year, with risk factors being rising levels of the serum paraprotein and an IgM or IgA isotype. A Mayo Clinic study determined that subjects with an IgM paraprotein developed a lymphoproliferative disorder at the rate of about 1.5% per year [2].

MGUS are typically 1-5g/l and almost always less than 9-10g/L). They are more frequently of IgG heavy chain and are typically not associated with suppression of normal immunoglobulin levels, nor with the presence of free light chains in the urine. Paraproteins associated with malignant disease, on the other hand, are present at higher concentrations (usually greater than 10g/L). If detected early when their initial concentration is low, a paraprotein's concentration tends to increase over time. They may be associated with other clinical abnormalities, cytopenias, depression of normal immunoglobulin levels and sometimes, the presence of free light chains in urine. Paraproteins in this category with an IgM heavy chain are associated with lymphomas, especially Waldenstrom's

macroglobulinaemia, or CLL, whilst IgG, IgA or IgD more frequently with myeloma. The presence of free light chains is much more common in myeloma than lymphoma and free λ light chains are also associated with amyloidosis.

IMMUNOLOGICAL INVESTIGATIONS FOR THE PRESENCE OF A PARAPROTEINS

Serum protein electrophoresis (EPG) and immunofixation (IFX) has a sensitivity of about 87% for the presence of a monoclonal paraprotein, whilst the serum free light chain assay (SFLC) has a sensitivity of about 74%. The combination of serum and urine EPG/immunofixation or SFLC and serum EPG/immunofixation each identifies about 97% of monoclonal paraproteins. A combination of SFLC, serum and urine EPG and immunofixation is said to detect about 99% of patients with serum paraproteins or urine free light chains (Bence Jones protein).

Thus, for maximum sensitivity in detection of paraproteins it is suggested that the following be ordered: IgG, IgA and IgM levels, serum and urine EPG and immunofixation, and SFLC.

Immunoglobulin (Ig) levels: This involves measurement of of IgG, IgA and IgM, most often in serum. Marked elevation of only a single antibody class, especially if associated with reduction of other immunoglobulins, suggests the possible presence of a paraprotein. Additionally, a marked decrease in immunoglobulin of all classes may indicate a free light chain-producing myeloma.

Serum EPG: The separation of proteins on the basis of their charge is the primary method for paraprotein identification. However serum paraproteins, especially of IgM or IgA isotype, can sometimes be missed on serum EPG. This occurs most frequently because of the co-migration of the paraprotein with bands of other major, normal serum proteins or because the paraprotein quantity is very low (generally less than 1g/L). EPG followed by immunofixation (see below) of serum is required to prove monoclonality and identify small or masked paraproteins.

Urine EPG: This test can detect monoclonal free light chains (Bence Jones protein) that may not be detectable on serum EPG. Intact immunoglobulin (heavy and light chain) is also sometimes found in urine EPG. As with serum EPG, abnormalities in urine EPG must be followed up by urine immunofixation to demonstrate monoclonality. With renal impairment, serum free light chains may also be readily detected in serum by immunofixation.

Immunofixation (IFX): A serum or urine sample subjected to electrophoresis is then separately incubated with antibodies for each of the heavy (G, M, A) and light (κ or λ) chains. A paraprotein will only react with a single heavy chain and a single light chain type (e.g. IgGκ), or only a single light chain class if only free light chains are present. IFX must be

carried out in order to confirm monoclonality if a putative paraprotein band is found on electrophoresis.

Serum free light chains (SFLC): The presence of a paraprotein results in excess production of free kappa or lambda light chains. In this assay, these free light chains are measured separately using polyclonal antibodies to light chain epitopes which are only visible to the antibody when they are unbound (free) of immunoglobulin heavy chains. This assay cannot identify heavy chains. The results are presented as individual light chain levels and as a kappa to lambda ratio, which is distorted in the presence of a paraprotein. These levels are also influenced by renal impairment which can complicate their interpretation.

For a more detailed discussion of this assay please see the separate information sheet entitled "Use of the serum free light chain assay (SFLC) assay for identification of monoclonal paraproteins".

References

1. Wadhera RK et al. Prevalence of monoclonal gammopathy of undetermined significance: a systematic review. Mayo Clin Proc. 2010;85:933-942.

2. Kyle RA et al. IgM monoclonal gammopathy of undetermined significance and smoldering Waldenstrom's macroglobulinemia. Clin Lymphoma Myeloma. 2009;9:17-18.