



Paraproteins: the clinical approach

Peter Mollee

Pathology Queensland

Princess Alexandra Hospital

Brisbane



Overview

- What is important to clinicians?
- Clinical perspective on aspects of electrophoresis reporting
 - ▣ Importance of response assessment, especially complete remission
 - ▣ Co-migrating paraproteins
 - ▣ Small bands post-transplant
 - ▣ First presentation of small bands
- Commenting recommendations



Serum and urine protein electrophoresis

- Primary role is to detect monoclonal immunoglobulins associated with plasma cell dyscrasias and lymphoproliferative disorders
- Also provides information regarding:
 - ▣ Acute phase response
 - ▣ Alpha-1 antitrypsin deficiency
 - ▣ Hyper- and hypo-gammaglobulinaemia



Serum and urine protein electrophoresis

- What do clinicians really want?
 - ▣ Is a paraprotein present?
 - ▣ How great is its concentration?
 - ▣ Cumulative reporting



Serum and urine protein electrophoresis

- What do clinicians also want?
 - ▣ Enough information to calculate response to therapy
 - ▣ Uniform approach to report paraproteins that co-migrate with normal serum proteins
 - ▣ Recognition and reporting of oligoclonal and small bands that occur post-transplant
 - ▣ Consistent reporting of paraproteins



Why is response assessment important?

- New more active agents brings need to assess not just if response has occurred, but the exact magnitude of response
- Complete response particularly important
- Assess need for and type of next treatment
- PBS requirement to obtain some drugs



Clinical perspective

- Myeloma management in 1990
 - ▣ Melphalan and prednisolone standard therapy
 - Complete remissions <5%
 - Partial remissions ~33%
 - ▣ Accurate response assessment less important as therapeutic options limited



Clinical perspective

- Myeloma management in 2010
 - ▣ Multiple therapeutic options
 - Conventional chemotherapy
 - Autologous and allogeneic stem cell transplantation
 - Thalidomide, lenalidomide, pomalidomide
 - Bortezomib, carfilzomib
 - Panobinostat, vorinostat
 - Multiple others under investigation
 - ▣ Improved response rates and survival



Clinical perspective

Regimen	Complete remission	1 yr OS
Melphalan + prednisolone (MP)	2%	70%
Autologous stem cell transplantation	~20%	80%
MP + thalidomide	16%	90%
MP + bortezomib	32%	92%
MP + lenalidomide	24%	100%
Lenalidomide + dexamethasone	~15%	94%
PAD/ASCT/lenalidomide	66%	100%



Assessment of response to therapy

- Depth of response important
 - ▣ CR, nCR and vgPR all strong prognostic markers
 - Predict event-free and overall survival
 - Goal of therapy, especially in younger patients
 - Achievement dictates further treatment

- Multiple publications highlighting important of response criteria
 - ▣ Myeloma: *Durie BG et al. Leukemia 2006;20:1467-73*
 - ▣ Waldenstrom's macroglobulinaemia: *Kimby E et al. Clinical lymphoma & myeloma 2006;6:380-3*
 - ▣ AL amyloidosis: *Gertz MA et al. American journal of hematology 2005;79:319-28.*

Protein electrophoresis and response criteria for multiple myeloma

Response category	Abbreviation	Response criteria	Comment
Complete remission	CR	Negative IFE of the serum and urine	IFE required if paraprotein not visible by electrophoresis
Near complete remission	nCR	Paraprotein visible by IFE but not on electrophoresis of the serum or urine	IFE required if paraprotein not visible by electrophoresis
Very good partial remission	vgPR	Paraprotein visible by IFE but not on electrophoresis of the serum or urine OR ≥ 90% reduction in serum paraprotein plus urine paraprotein <100 mg/24 h	IFE required if paraprotein not visible by electrophoresis
Partial remission	PR	≥ 50% reduction of serum paraprotein and reduction in 24-h urinary paraprotein by ≥ 90% or to < 200 mg/24 h	Allow use of quantitative immunoglobulin levels in patients in whom the paraprotein measurements are unreliable (e.g. IgA paraproteins co-migrating with the beta region)



Serum and urine protein electrophoresis

- What do clinicians also want?
 - ▣ Enough information to calculate response to therapy
 - ▣ **Uniform approach to report paraproteins that co-migrate with normal serum proteins**
 - ▣ Recognition and reporting of oligoclonal and small bands that occur post-transplant
 - ▣ Consistent reporting of paraproteins



Paraproteins co-migrating in the non-gamma region e.g. IgA in the beta region

- Both densitometric paraprotein and immunochemical total immunoglobulin measurements have limitations
 - ▣ Densitometry measures both the paraprotein and the co-migrating proteins
 - Low level paraproteins not reliably measured
 - Subtracting the beta region from the total fractionation is inherently unreliable
 - ▣ INA/ITA methods measure both the paraprotein and polyclonal immunoglobulin of that isotype
 - May overestimate immunoglobulin values at higher concentrations because of non-linearity
- Densitometric and immunochemical measurements cannot be used interchangeably

But, clinicians need some information for decision making ...



Paraproteins co-migrating in the non-gamma region e.g. IgA in the beta region

□ Solution

- Laboratories should support reporting of both the densitometric paraprotein and INA/ITA immunoglobulin measurement
- Report the total protein in the beta region (beta+paraprotein) quantitation at presentation and during monitoring
- Report relevant total immunoglobulin
- Comment to reflect uncertainty e.g. “IgA paraproteins in the beta region may not be reliably quantitated by either electrophoresis or measurement of total immunoglobulins. Both methods can be useful in monitoring paraproteins depending upon the clinical circumstance”



Serum and urine protein electrophoresis

- What do clinicians also want?
 - Enough information to calculate response to therapy
 - Uniform approach to report paraproteins that co-migrate with normal serum proteins
 - **Recognition and reporting of oligoclonal and small bands that occur post-transplant**
 - Consistent reporting of paraproteins



Small bands after stem cell transplantation

- Small abnormal protein bands are frequently seen on SPEP following transplantation
- Often isoelectric focussing reveals not only oligoclonal bands but small discrete bands with the appearance of a paraprotein
 - ▣ Typically ≤ 1 g/L but may occasionally be larger
 - ▣ Persist from between one to 18 months
- Likely due to transient dysregulation of the regenerating B cell compartment during haematopoietic recovery



Small bands after stem cell transplantation

- May be mistakenly reported to suggest relapse
 - ▣ Associated with improved remission depth and outcome
- Also described following novel agent combination regimens

Laboratory reporting

- Recognise these small bands
- Don't report as new paraproteins
 - ▣ Avoid terms such as “paraprotein” or “monoclonal protein”



First presentations of small bands

- High resolution SPEP detects 0.5-1 g/L size bands
 - ▣ Clinical significance often uncertain
- Not reporting these small bands risks misdiagnosis of important diseases
 - ▣ AL amyloidosis
 - ▣ Oligosecretory myeloma
 - ▣ Lymphoma



First presentations of small bands

- Small bands often due to infectious and autoimmune diseases
 - ▣ Overcalling these small bands can precipitate a cascade of investigations because of clinician concern that such small bands indicate clonal plasma cell or lymphoproliferative disease
- **Solution**
 - ▣ Note that a small band (rather than a paraprotein or monoclonal immunoglobulin) is present
 - ▣ Comment that its significance is not known
 - ▣ Suggest urine testing to exclude BJP
 - ▣ Suggest follow-up testing

Commenting Recommendations – paraprotein and small band samples

Pattern	Minimal comment
First detection of a paraprotein	Suggest total serum immunoglobulins and urine protein electrophoresis and immunofixation (if not already done/ordered). [Typing and numerical quantitation e.g. “An IgG kappa paraprotein of 10 g/L was detected in the gamma region”]
Follow up of a known paraprotein which is still present	Nil required [A comment should be made on the original band and its current status e.g. “The previously reported IgG kappa paraprotein now measures 5 g/L”]
Paraprotein detected only by immunofixation electrophoresis	The previously reported IgG kappa paraprotein is now only visible by immunofixation
If paraprotein has disappeared	A comment is required to confirm the absence of the previously detected paraprotein e.g. “The previously reported IgG kappa paraprotein was not detected by immunofixation”



<u>Serum protein electrophoresis</u>		<u>Unit</u>	<u>Ref</u>
Total Protein	85	g/L	
Albumin	22	g/L	
Total Globulin	63	g/L	
Paraprotein	DET		
Alpha-1	3	g/L	
Alpha-2	8	g/L	
Beta	8	g/L	
Gamma	44	g/L	
IgG kappa paraprotein	41	g/L	
Monoclonal kappa FLC	Trace	g/L	
Residual gamma	3	g/L	
IMMUNOGLOBULINS:			
IgG	45	g/L	
IgA	0.5	g/L	
IgM	0.2	g/L	

DET, detected; ND, not detected; SEEC, see comment; FLC, free light chains

Comments:

Decreased residual gamma globulins. Suggest urine protein electrophoresis and immunofixation.



Commenting Recommendations – paraprotein and small band samples

Pattern	Minimal comment
First detection of a paraprotein	Suggest total serum immunoglobulins and urine protein electrophoresis and immunofixation (if not already done/ordered). [Typing and numerical quantitation e.g. “An IgG kappa paraprotein of 10 g/L was detected in the gamma region”]
Follow up of a known paraprotein which is still present	Nil required [A comment should be made on the original band and its current status e.g. “The previously reported IgG kappa paraprotein now measures 5 g/L”]
Paraprotein detected only by immunofixation electrophoresis	The previously reported IgG kappa paraprotein is now only visible by immunofixation
If paraprotein has disappeared	A comment is required to confirm the absence of the previously detected paraprotein e.g. “The previously reported IgG kappa paraprotein was not detected by immunofixation”



<u>Serum protein electrophoresis</u>		<u>Unit</u>	<u>Ref</u>
Total Protein	85	g/L	
Albumin	22	g/L	
Paraprotein	DET		
Paraprotein 1	41	g/L	
Paraprotein 2	Trace	g/L	
IMMUNOGLOBULINS:			
IgG	45	g/L	
IgA	0.5	g/L	
IgM	0.2	g/L	

DET, detected; ND, not detected; SEEC, see comment; FLC, free light chains

Comments:

Paraprotein 1: An IgG kappa paraprotein was detected in the gamma region
Paraprotein 2: A monoclonal kappa FLC was detected in the gamma region.
Decreased residual gamma globulins. Suggest urine protein electrophoresis and immunofixation.



Commenting Recommendations – paraprotein and small band samples

Pattern	Minimal comment
First detection of a paraprotein	Suggest total serum immunoglobulins and urine protein electrophoresis and immunofixation (if not already done/ordered). [Typing and numerical quantitation e.g. “An IgG kappa paraprotein of 10 g/L was detected in the gamma region”]
Follow up of a known paraprotein which is still present	Nil required [A comment should be made on the original band and its current status e.g. “The previously reported IgG kappa paraprotein now measures 5 g/L”]
Paraprotein detected only by immunofixation electrophoresis	The previously reported IgG kappa paraprotein is now only visible by immunofixation
If paraprotein has disappeared	A comment is required to confirm the absence of the previously detected paraprotein e.g. “The previously reported IgG kappa paraprotein was not detected by immunofixation”



Commenting Recommendations – paraprotein and small band samples

Pattern	Minimal comment
Paraprotein in the beta region	IgA paraproteins in the beta region may not be reliably quantitated by either electrophoresis or measurement of total immunoglobulins. Both methods can be useful in monitoring paraproteins depending upon the clinical circumstance.
Small abnormal band with different electrophoretic mobility from the original paraprotein in a patient with a known paraprotein	There is a small (type: e.g. IgG kappa) band, approximately (amount: e.g. 1 g/L) on a background of a polyclonal and/or oligoclonal pattern. This band is different from the original paraprotein. Its clinical significance is uncertain.
First presentation of small abnormal band	There is a small (type: e.g. IgG kappa) band approximately (amount: e.g. 1 g/L). Its clinical significance is uncertain. Suggest urine electrophoresis and immunofixation (or serum free light chains) and repeat serum protein electrophoresis (in 3 to 6 months) if clinically indicated.