Detection of Gammopathy by Capillary Zone Electrophoresis (CZE) and two-dimensional electrophoresis (2-DE) in patients with post-transplantation lymphoproliferative disease

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BACKGROUND

Post-transplantation lymphoproliferative disease (PTLD) is an uncommon but serious complication of immunosuppression following solid organ and hematopoietic stem cell transplantations. The majority of PTLD results from Epstein-Barr virus (EBV)-driven B cell proliferation poorly controlled by a weakened immune response. PTLD can be classified into several clinicopathological groups ranging from polyclonal lymphoid hyperplasia harboring a polymorphic histological pattern, to monoclonal aggressive non-Hodgkin B cell lymphomas with monomorphic histological pattern with poor prognosis.
Treatment efficacy is usually monitored by imaging parameters, which lack sensitivity. Thus, new tools for monitoring PTLD are needed. It has been reported that gammopathy detection in sera is associated with PTLD development following liver transplantation, and that the evolution of such gammopathies might be predictive for PTLD evolution.

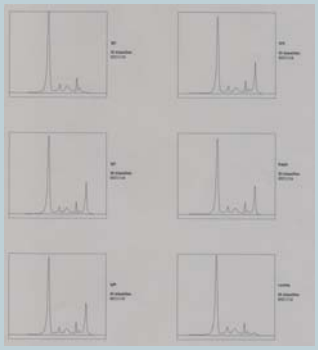
STUDY DESIGN

A retrospective study was conducted to:

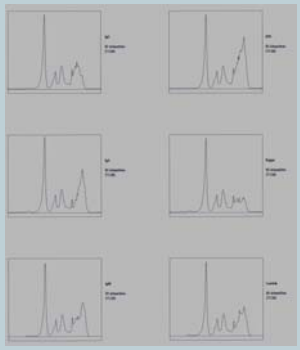
1. compare sensitivities and specificities of two-dimensional electrophoresis (2-DE) with those of capillary zone electrophoresis (CZE) with immunosubstraction for detecting serum monoclonal immunoglobulins in patients with PTLD,
2. establish a proteomic map representative of PTLD patients,
3. compare such a map with that obtained in patients candidates for transplantation and without PTLD.

Serum samples from 7 patients (1 female and 6 males, 17 to 68 years old) with PTLD following transplantation of kidney (n = 5), heart (n = 1) and liver (n = 1) were analyzed. Samples were taken at time of PTLD diagnosis. Whenever applicable, results were compared with those from additional sera obtained from the same patients before transplantation (n = 4), and from patients candidates for transplantation and without PTLD (n = 3).

The median time from transplantation to PTLD was 4.1 years (range: 4 months to 17 years), with early PTLD (< 1 year) occurring in 4 patients. B-cell PTLD were subclassified by histology and molecular biology techniques into monomorphic monoclonal type (n = 6), polymorphic monoclonal type (n = 3) and polymorphic oligoclonal type (n = 1). Tumors were associated with EBV in 7 cases.

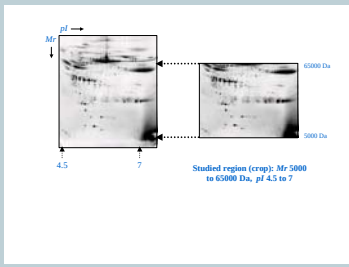


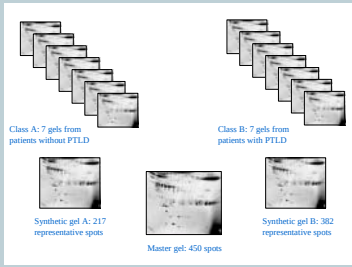
CZE of a monoclonal IgG λ gammopathy



Oligoclonal aspect of CZE in patient # 2

Patient #	Age (year) / sex	type of transplant	Time from transplantation to PTLD diagnosis (year)	Histology classification of PTLD	Clonality of PTLD	EBV in PTLD
1	17 / M	liver	12	monomorphic	monoclonal	present
2	26 / M	kidney	0.3	polymorphic	oligoclonal	present
3	64 / M	kidney	3	polymorphic	monoclonal	present
4	36 / M	heart	17	monomorphic	monoclonal	absent
5	53 / F	kidney	0.4	monomorphic	monoclonal	present
6	32 / M	kidney	3	polymorphic	monoclonal	absent
7	68 / M	kidney	12	monomorphic	monoclonal	absent
8	59 / M	kidney	0.16	polymorphic	monoclonal	present
9	66 / F	liver	0.5	monomorphic	monoclonal	present
10	67 / M	AHSC	2	monomorphic	monoclonal	present





METHODS

* **CZE with immunosubstraction**: was performed with the Paragon CZE 2000 clinical capillary electro-phoresis system. Protein separation was realized during 5 min at 8000 V and 24°C in fused-silica capillaries and detected by direct light absorption at 214 nm through a small optical window. Serum samples were incubated with γ, α, μ, κ and λ antibodies bound to sepharose beads. After beads settled down, a second capillary electrophoresis was performed on the supernatant. Electrophoregrams realized before and after incubation with specific immunobeads were compared and immunotyping determined by the absence of the specific peak.

* **Sample preparation**: 5 µl of serum were mixed with 10 µL of a solution containing 20 % SDS and 0.3 M DTE, samples were heated to 95°C for 5 minutes and then diluted to 500 µL with a solution containing 9M urea, 4% CHAPS, 65 mM DTE and 5% pH 3-10 ampholytes. 50 µl of the mixture were loaded onto the first dimension gels.

* **2-DE**: isoelectric focusing (IEF) was performed using non-linear immobilized pH gradient (Immobiline Dry Strip pH 3-10, 18 cm from Amersham Pharmacia), with sample cup loading. For the second dimension, proteins were separated on 9-16% gradient polyacrylamide gels using diacrylylpiperazine as crosslinking agent. Proteins were visualized by silver staining.

*** Bioinformatic analysis**: the silver stained gel images were captured with high-resolution densitometry reading with the Personal Laser Densitometer (Amersham Pharmacia Biotech). Informatic analyses were performed using the software Melanie 3.0 (Genebio), spots were detected automatically. Manual spot editing (ie, separation of 2 spots), or deleting (artefacts) were performed when necessary. Alignment and matching of the spots were realized by choosing one gel as reference and manually selecting several common spots as landmarks. These ones were selected in order to be spread out through the studied areas. The size as well as the intensity of each detected spots were expressed in % volume.

Bioinformatic analysis was focused on the region of the gels corresponding to pI between 4.5 and 7, and Mr between 5'000 and 65'000 daltons. The approach used to study variations in protein expression was to produce synthetic and master gels by merging spots from these gels. The master gel contained all spots representative of each class of samples, and was used for statistical analysis.

In order to generate the master gel, synthetic gels were created, one per class of samples. For the synthetic gel, seven samples of each class were matched one to another, using three gels as reference ones. The synthetic gels contained the spots present on at least three gels of each class.

RESULTS :

* **CZE**: with immunosubstraction revealed no gammopathy in controls and in 7 PTLD patients, an oligoclonal immunoglobulin (Ig) pattern in 1 patient (polymorphic oligoclonal type), and a monoclonal Ig pattern in 2 PTLD patients (monomorphic monoclonal type / polymorphic monoclonal type).

* **2-DE**: no monoclonality observed on 2-DE gels of samples with and without PTLD.

* **Bioinformatic analysis with 2-DE**:

1. **Class A**: samples of patients without (yet) history of PTLD (taken before transplantation), 7 gels with 523 to 743 spots resulting synthetic gel with 217 representative spots
2. **Class B**: samples with history of PTLD (taken at moment of diagnosis), 7 gels with 601 to 851 spots resulting synthetic gel with 382 representative spots
3. The master gel contained 450 spots present on 2 synthetic gels, of which 149 spots were common,
4. From these 450 spots, 450 histograms were created for analysis: no characteristic spots for PTLD found after bioinformatic analysis (= no significant changes in spot volume between the 2 classes of samples).

CONCLUSIONS :

CZE with immunosubstraction helps to diagnose PTLD, but its sensitivity appears low. 2-DE analysis does not further help for evidencing gammopathy associated with PTLD. Furthermore, this latter method did not evidence a typical protein map pattern with PTLD.