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A new immune-toxicological test for polysulfone hypersensitivity in hemodialysis patients

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Abstract

Incompatibility of dialysis procedure due to hypersensitivity against dialyzer material, currently mainly based on polysulfone and derivatives, cannot be assessed by routine laboratory tests. Although the frequency of such symptoms is suspected to be lower than 2%, it resembles an important clinical problem because dialysis procedures are frequently accompanied by symptoms of non-tolerability with reasons not being entirely clear. To enlighten the role of polysulfone hypersensitivity, we adapted known standardized material immune-toxicological tests (lymphocyte transformation test, basophil degranulation test) to the specific conditions of dialysis and polysulfone material sensitivity. We developed a method of polysulfone micronisation and measured humoral immune response of isolated patient's lymphocytes when incubated with polysulfone dispersion. Thirty-nine samples from 103 patients with suspected polysulfone hypersensitivity within the dialysis population of a nation-wide dialysis provider (n = 15.761 patients) showed positive results for type 1 (n = 19), type 4 (n = 18) or both type (n = 2) reactions. This is the first methodological report showing plausible in-vitro results of patients' samples concerning polysulfone intolerance. Further clinical and laboratory research is needed to define true polysulfone hypersensitivity and to enlighten the field of hypothetic subclinical material incompatibility in patients with impaired dialysis tolerability.

Keywords

Hypersensitivity, hemodialysis, lymphocyte basophil transformation test, degranulation, type 1, type 4

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Background

Biocompatibility refers to the sum of specific interactions between blood and the artificial materials of the hemodialysis (HD) circuit. Because the components of the HD procedure are non-self,[1,2] the interaction of blood with these components causes an "inflammatory response." When this response is mild and well tolerated, the material can be termed biocompatible. When it is intense, it may adversely affect patient well-being or lead to deleterious outcomes.

These inflammatory responses have clinical consequences that range from overt acute hemodynamic instability to more diffuse and less well-defined states of disease. Symptomatic incompatibility of polysulfone (PS) dialysis materials has been a problem that is so far difficult to address. Except for clinically clear type I reaction according to Gell and Coombs[3,4] (anaphylaxis with dyspnea, urticaria and blood pressure drop), there are currently no clear clinical symptom constellations or validated laboratory tests as evidence of a PS allergy against dialysis materials. A HD-associated hypersensitivity reaction can manifest itself as of anaphylactic (type I, Coombs & Gell) and/or non-anaphylactoid origin. Effector cells (e.g. mast cells and basophils) can be

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activated through cross-linking of FcεRI-bound specific IgE (high-affinity lgE receptor (Fc epsilon(ε) RI))[5,6] leading to rapid release of preformed mediators (e.g. histamine, heparin) or slower de novo synthesis and release of arachidonic acid metabolites (such as sulfidoleukotriens (sLT) and prostaglandin D2) and cytokines (e.g. IL-4). Effector cells can also be triggered without IgE involvement (so-called anaphylactoid reactions) directly via the anaphylatoxins C3a and C5a. An anaphylactic material reaction (type 1) is generally characterized by a triad of blood pressure drop with shock, urticaria, and dyspnea. In everyday clinical practice, however, there are usually rather minor symptomatically mixed forms that also contain elements of a late reaction (type 4) and often do not allow a clear assignment.

Currently, warning alerts have been published in Spain and Germany by medical safety authorities belonging to suspected growing incidences of such hypersensitivity reactions. In Spain, a recent epidemiological paper found 37 patients out of 1561 (2.37%) patients having hypersensitivity reactions.[7] The membranes involved were polysulfone (n = 23), polynephron (n = 8), polyethersulfone (n = 1) and polyacrylonitrile (n = 1). Within the largest non-profit dialysis provider in Germany, among 15.761 prevalent dialysis patients, 379 were reported with suspected PS hypersensitivity with the consequence of providing alternative, non-PS membranes for dialysis.[8] Further knowledge about incidence is basically available through case reports or small series.[9–13] Specific laboratory tests, as were available for other types of material insensitivity[14–16] are currently not on the market.

For a more pathophysiological evaluation, we adapted formerly described specific immune-toxicological tests[17,18] to the special need of HD material hypersensitivity assessment and provide the initial results of this new PS hypersensitivity test system.

Material and methods

Heparinized blood samples from patients with suspected PS hypersensitivity were drawn before and after hemodialysis procedures. Patients with suspicion of PS sensitivity were drawn from a distribution list of a nation-wide dialysis organization (Kuratorium for Dialysis and Transplantation, KfH), showing supply of non-PS membranes. The overall number of HD in-center patients cared by KfH centres in 2019 was 15,761. Of these, 395 patients were provided with non-PS membranes following PS hypersensitivity suspicion.[8] Of 395 suspected patients, 103 provided blood for analysis after provider-intranet-based information of their nephrologists about the new availability of a routine PS sensitivity test, which was reimbursed by health care insurances. Analysis of hypersensitivity was performed by in-vitro exposure of patients' lymphocytes against a prepared PS dispersion. Blood lymphocytes were isolated by Ficoll's gradient separation and incubated with PS of the suspicious specific dialyzer material. That dispersion was provided by micronisation of the respective PS material directly from non-used fresh dialyzers.

Detection of type I reactions was performed by assessment of the basophil degranulation test (BDT) and subsequent measurement of sulfidoleukotrienes, histamine and 5-hydroxytryptamine and quantification with immunoassay tests.[17–19] The quantification of released sLT's after incubation of PS dispersion with isolated basophils in comparison to a negative control (without antigenic material) of the same patient serves as a diagnostic tool for eliciting HD-induced hypersensitivity. Results (Figure 1 right panel) are given as degranulation indices (DI, %) in relation to positive controls. An increase of >250 pg/mL greater than the value of the negative probe was considered a positive result.

Detection of type IV reactions were performed by lymphocyte transformation test (LTT) and measurement of the proportion of specifically stimulated lymphocytes (specif. lymphogenesis). For such effects, effector lymphocytes were incubated with PS dispersion followed by measurement of IFN_{γ} and IL-5 using ELISA (type IV a / b).[20–22] Total quantity of released sLT's after incubation of effector cells with anti-FcεRI-antibody combined with the chemotactic peptide N-formyl-L-methionyl-L-leucyl-L-phenylalanine (fMLP) with the one after incubation with dialyser material (minus background values), whereby a quantity of $\geqslant$50% of totally released sLT's in the probe signifies a statistically significant positive test result (DI $\geqslant$ 50%), with progressive borderline values between 20% and 49% (DI $\geqslant$ 20% $<$ 50%) and a negative result when only $<$20% of the total released sLT's could be measured (DI $<$ 20% = negative). Individual results (Figure 1 right panel) are given as stimulation indices (SI, proliferation rate with/proliferation rate without antigen; positive SI $\geqslant$ 3), and ratio of stimulated to unstimulated cells, LyGenes, %, cutoff $\geqslant$ 10.0%) in relation to positive controls.

After the laboratory investigations, fully anonymized data, but containing the brand-type PS dialyzer, were subjected to statistical analysis of the in vitro cellular exposure results (SPSS v22, IBM).

Results

Fifty-four lymphocyte isolates from 103 patients with clinically suspected PS hypersensitivity showed positive examination results in BDT (type 1) and/or LTT/spec. lymphogenesis (type 4) either before and/or after dialysis (type 1 tests 7 before, 9 after HD and 8 unspecified time; type 4 tests 11 before, 11 after HD, 8 unspecified time point). Because not for all patients' samples were available before and after dialysis, the frequency of any positive samples (before or after dialysis or unspecified)

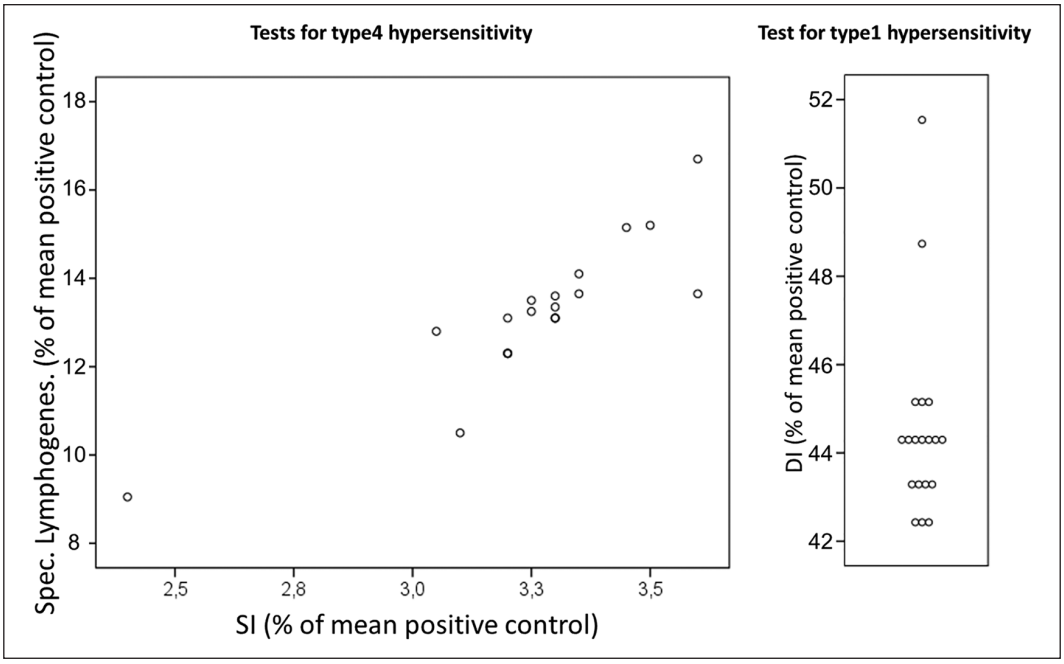

Figure 1. Correlation of stimulation assays. Left panel shows results of type 4 tests LTT and specif. lymphogenesis. Right panel shows results of the applied type 1 test BDT.

Table 1. Result of stimulation assays by dialyzer material.

	Polysulfone n = 26	Polyethersulfone n = 47	Polyethersulfone/ polyamid n = 26	Vitamin-E-coated PS n = 1	Polysulfone/ polyvinylpyrrolidon n = 2
DI (% of mean)	44.4 ± 2.13	44.4 ± 3.0	44.0 (1 pos.)		44.5
SI (% of mean)	3.34 ± .152	3.27 ± .095	3.31 ± .197	2.40	
Lymphog. (%)	13.8 ± 1.61	13.2 ± 1.04	13.3 ± 1.45	9.05	
Pos. Type 1 (n, %)	*8*, 31	9, 19	1, 4		1
Pos. Type 4 (n, %)	*5*, 19	7, 15	7, 27	1	

PS: polysulfone; DI: degranulation indices; SI: stimulation indices.

was assessed Using any available positive threshold, 19 samples were positive for BDT (type 1) and 20 for LTT/ specif. lymphogenesis (type 4). In only 2 patients, positive type 1 and type 4 results were observed in the same lymphocyte isolate. Results by different dialyzer PS materials (polysulfone (Fx™ series FMC), polyethersulfone (Elisio™ series Nipro), polyacrylethersulfon/polyamid (Polyflux™ Gambro), Vitamin-E-coated polysulfon (Vie18A™ Asahi), polysulfon/polyvinylpyrrolidone (Cordiax™ series FMC), PMMA (Filtryzer™ Toray)) are given in Table 1.

The time point of taking blood was not significant, because in patients with samples before and after dialysis, no significant differences of DI, SI and lymphogenesis before and after dialysis (average delta -0.4; -0.28; - 1.74, $p = 0.71$; 0.34; 0.37) and with different dialyzer materials (Table 1) were observed. Patients with positive type 4 results (LTT and lymphogenesis) showed highly correlated results in either LTT or lymphogenesis test (Figure 1, $R = 0.87$, $p < 0.0001$). Eight out of 8 samples from patients with repeated test on different PS showed positive results on either PS. One patient tested positive on PS showed no hypersensitivity with another non-PS (PMMA) material.

Discussion

In this methodological study, stable and reproducible positive stimulation results of isolated human lymphocytes after exposition against that dialyser material which was supposed to cause hypersensitivity were obtained in a substantial rate of all investigated samples (39%). The numerical results were all mapped into a range of 40%–50% cellular reactivity of that of in vitro positive controls indicating a sufficient positivity based on the predefined methodology thresholds. On the first superficial view, a ‘false-negative’ rate of 60% looks rather disappointing,

because all samples derived from patients with suspicion of PS hypersensitivity. However, due to the clinical variability of intolerance symptoms and the high prevalence of any problems after HD initiation, mainly of circulatory origin after initiating extracorporeal circuit, this rate may obviously express the true frequency of isolated PS material hypersensitivity in suspected patients. This hypothesis needs further investigation by detailed clinical characterisation of affected patients' symptoms and study of a negative, non-PS-hypersensitive cohort. Alternative pathophysiological pathways of material sensitivity like complement activation[23,24] remain to be elucidated and incorporated into a comprehensive future testing panel. As such, the effect of HD on eosinophilia induction to generate cytokines is well known[25] and needs to be taken into future consideration.[26] Eosinophilia can produce a multitude of inflammation and fibrosis factors that cause heart and nerve damage and thrombosis.[27] Moreover, the role of mild incompatibility without causing indicative clinical symptoms and hypothetically contributing to not-well tolerated dialysis has not been understood. To enlighten this field, looking into relationships between symptoms of subclinical dialysis non-tolerability and highly sensitive material reactions would be helpful. When analysing our current results, it looks that the applied methods provide highly specific, but not so much 'over'-sensitive results.

Not only in that sense, our finding underlines the importance of a clinically accurate characterization of such complications and the need for further scientific investigation with specific regard on reproducibility and re-evaluation after change to alternative materials. The lack of these clinical data represents the most important limitation of our study having in mind that non-availability of a 'golden standard' vs in vitro results is a typical situation in toxicological testing. Therefore, of note, all applied tests were accompanied by in vitro positive controls that confirmed the test reliability.

If our testing results represent the true PS hypersensitivity rate in patients of the underlying dialysis provider, PS allergy would occur with a prevalence of about 100 hyper-sensitive in 100,000 non-sensitive patients (0.1 %) which is lower compared with numbers and rates reported in Spain.[7] But again, due to the complexity of non-compatible dialysis symptoms, no standardized and inter-comparable incompatibility rates can be given at the current stage of research.

In positive samples, no differing results before or after dialysis were observed in either test. The tests for type 4 reactions (LTT and specif. lymphogenesis) were highly correlated. The 100% confirmation rate of positivity in a subgroup with repeated measurements with alternative PS is another indication of the technical method reliability.

Therefore, we suggest that one single blood sample 10 min after dialysis initiation exposed to one type 4 and one type 1 test is sufficient to yield results helpful to determine the meaning of sometimes highly variable clinical symptoms.

In summary, we present a new test based on the immunological stimulation answers of isolated patients' lymphocytes after exposure against suspected hypersensitizing dialyzer PS material. Such test can be used for deeper investigation of clinical suspicious PS hypersensitivity.

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Author contributions

JB, PR: Idea, study design, data analysis, manuscript writing, manuscript revision. PR: Laboratory analysis. RW and DR: Manuscript writing, manuscript revision.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Disclosure statement

KPR is founder and co-owner of the laboratory, which performed the test described in the manuscript.

Ethical approval

The work presented in that manuscript complies with guidelines for human studies. Because anonymized test results from routine care and containing no patient data were studied, no ethical approval for this non-invasive descriptive study was gained.

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