LONG-TERM PERITONEAL DIALYSIS TREATMENT PROVOKES ACTIVATION OF GENES RELATED TO ADAPTIVE IMMUNITY

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Running title: *Immune response during PD treatment*

Summary

Permanent irritation of the peritoneum during peritoneal dialysis (PD) treatment leads to local chronic inflammation and subsequently activation of processes driving fibrogenesis in the long-term. The aim of the study was to compare the peritoneal effluent transcriptome of 20 patients treated less and 13 patients treated more than 2 years using microarray analysis. An increased expression of genes associated with an immune response was observed in long-term treated patients with well preserved peritoneal function, when compared to patients treated less than 2 years. From 100 genes highly expressed in long-term patients, a significant up-regulation of six was found by RT-qPCR: *LY9* (lymphocyte antigen 9), *TNSFR4* (tumor necrosis factor receptor superfamily, member 4), *CD 79A* (CD79a molecule), *CCR7* (chemokine C-C receptor 7), *CEACAM1* (carcinoembryonic antigen-related cell adhesion molecule 1) and IL2RA (interleukin 2 receptor alpha chain). Furthermore, the effluent cell population was analysed. A positive relationship between the number of granulocytes and NK cells on one hand, and duration of PD treatment on the other, was shown. We conclude, that the mechanisms of adaptive immunity promoting T helper 2 cells response are activated in the long-term before functional alterations develop. It consequently might trigger the fibrosis promoting processes.

Key words

Peritoneal dialysis, gene expression, peritoneal membrane alterations

Introduction

Peritoneal dialysis treatment is accompanied with alterations of the peritoneal membrane. Chronic inflammation resulting in angiogenesis and fibrogenesis has been suggested to be the key moment in the development of peritoneal membrane changes [Davies 2014]. Fibrogenesis is a complex process, primarily mediated by activated myofibroblasts producing collagen. Different regulators of individual importance are entangled. Of immune cells, mainly macrophages, but also T cells are involved in the pro-fibrotic response through cytokines release [Aufricht *et al.* 2017]. Interleukin 6 (IL-6) is secreted by T cells and macrophages within an immune response to specific microbial stimuli to resolve infection and local tissue damage. This cytokine plays a pivotal role in the switch from innate immune response to an adaptive immune reaction [Aufricht *et al.* 2017]. In experimental model of sustained innate inflammatory activation, IL-6 subsequently triggers Th1 cell-mediated altered immune response, thereby promoting fibrosis via regulation of matrix metalloproteinases [Fielding *et al.* 2014]. Activated Th2 lymphocytes show a pro-fibrotic potential through cytokine release, which stimulates collagen production and fibroblast to myofibroblast differentiation [Pesce *et al.* 2006]. B cells might also be involved in fibrosis by regulation of T cell activation and IL6 release as a consequence of their antigen-presenting and co-stimulatory capacity [Hasegawa *et al.* 2005]. Development of fibrosis is accompanied by neoangiogenesis due to release of various pro-angiogenic chemokines and growth factors also involved in fibrotic processes [Wynn 2008].

The balance between ongoing chronic inflammation, tissue remodelling and tissue repair affects the development of peritoneal membrane alterations and might be different depending on the duration of PD treatment. The aim of the present study was to elucidate

genes potentially involved in peritoneal alterations during PD treatment by comparing the transcriptome of peritoneal cells in short- and long-term PD patients.

Patients and methods

The long-dwell PD effluents (median duration of 9 hours, range 8-14) of the dwell performed with a glucose based dialysis solution were acquired from 33 stable patients (21 males, 12 females) and centrifuged to obtain peritoneal cells for gene expression profiling using microarray analysis and for flow cytometry analysis. A 4-hour 3.86% glucose peritoneal equilibration test (PET) was performed on the day after the long-dwell collection in all patients. The patients showed no signs of infection at the time of examination and were peritonitis free at least 4 weeks preceding the test. Afterward, microarray results were validated in a larger cohort of 57 patients (validation set). The study protocol was approved by the Ethics Committee of the Institute for Clinical and Experimental Medicine.

Eight patients were treated with automated peritoneal dialysis (APD), the others with continuous ambulatory peritoneal dialysis (CAPD). All of them used commercially available glucose based dialysis solutions (Dianeal®, Baxter Healthcare Ltd., IRL-Dublin, Ireland). All but three used 7.5% icodextrin (Extraneal®, Baxter Healthcare Ltd., IRL-Dublin, Ireland) for the long dwell.

The patients were divided into a short-term PD group with a duration of PD treatment between 0 and 24 months and into a long-term one with a PD duration ≥ 25 months.

Peritoneal equilibration test (PET)

The PETs were performed during a four hours dwell with a 3.86% glucose dialysis solution (Dianeal®) under standardized conditions as described previously [Smit *et al.* 2003]. The test

itself was preceded in all patients by a rinsing procedure with fresh 1.36% glucose dialysis solution to avoid a possible effect of residual peritoneal volume before the test, on calculations of transport parameters. During the PET temporary drainage after 1 hour for assessment of free water transport was performed [Cnossen *et al.* 2009]. Blood samples were collected at 60, 120, 240 minutes. The dialysate samples were taken at multiple time points after inflow of the test solution: 0, 60, 120 and 240 minutes).

The dialysate over plasma ratio of creatinine (D/P_{creat}) and ratio of glucose concentration at 240 minutes over that at start of the dwell (D/D_0) glucose were assessed. Net ultrafiltration (NUF) is the difference between the in-situ intraperitoneal volume and the initial one. The fluid transport through the small pores ($SPFT_{60}$) was computed by dividing the amount of transported sodium within first hour with the plasma sodium concentration. The fluid transport through the small pores was then subtracted from the net ultrafiltered fluid volume after one hour, resulting in free water transport within first 60 minutes (FWT_{60}).

Gene expression profiling

Gene expression profiling of peritoneal cells was analysed using microarray on Illumina Human HT-12 v4 Expression BeadChips.

Peritoneal cells were obtained from peritoneal effluent of the long dwell preceding the PET by five consecutive centrifugations of 50 ml at 5000 g, 5°C for 20 minutes and stored at -20°C in lysis buffer until RNA isolation. Total RNA was isolated from using the RNeasy Micro Kit (Qiagen, Hilden, Germany) according to the manufacturer´s instructions.

Only RNA samples with an RNA integrity number > 6.5, as measured using the Agilent RNA 6000 Nano Kit on the Agilent 2100 Bioanalyzer (Agilent Technologies) were used for microarray analysis. A total of 200 ng of purified RNA served as a template for the

amplification and biotinylation of cRNA using the Illumina® TotalPrep™ RNA Amplification Kit, according to the manufacturer´s instructions. Yields of labeled cRNA were determined using the Agilent RNA 6000 Nano Kit on the Agilent 2100 Bioanalyzer (Agilent Technologies). Labeled cRNA (950 ng) was hybridized at 58 °C for 16 h to Illumina HumanHT-12 v4.0 Expression BeadChips (Illumina, Inc., San Diego, CA), and then washed according to the manufacturer's instructions. After hybridization, the chip was scanned using the BeadStation 500 instrument (Illumina), and raw data were extracted with the BeadStudio Data Analysis Software (Illumina). The R software lumi package was used to process the raw data. The quantile method was used for normalization.

Flow cytometry analysis

Leukocyte subtypes within peritoneal cells where phenotyped by a NAVIOS flow cytometer (Beckman Coulter, Brea, CA). The expression of the surface markers CD45, CD3, CD4, CD8, CD19, CD16, CD56 and, CD14 was examined using DuraClone IM Phenotyping Basic tubes with dried antibody panels (Beckman Coulter, Brea, CA) and the data were processed by Kaluza software (Beckman Coulter, Brea, CA). B cells were identified as CD45+CD19+; T cells as CD45+CD3+; CD8 cytotoxic T cells as CD45+CD3+CD8+; CD4 T helper cells as CD45+ CD3+CD4+; natural killer (NK) cells as CD45+CD3-CD56+; monocytes as CD45+CD14+ and granulocytes as CD45+ versus side scatter integral.

Measurements

Plasma and effluent concentrations of urea and creatinine were measured by enzymatic methods on an automated analyzer (Abbott Architect analyzer: Abbott Laboratories, Abbott

Park, Illinois, USA). Sodium concentrations were determined using the indirect ion selective electrode method. Glucose was measured on an autoanalyzer (Abbott Architect analyzer: Abbott Laboratories, Abbott Park, Illinois, USA) by the enzymatic hexokinase assay.

Statistical analysis

Data are presented as medians and ranges, unless stated otherwise. The Mann-Whitney U test was used to compare the transport parameters and AR of the short-and long-term PD patients due to asymmetrically distributed data. Spearman's rank correlation coefficient was used to examine possible relationships. All statistical analyses were performed using GraphPad Prism5.

Regarding gene analysis, only probes with average expression > 3.5 were used for further analysis. Differentially expressed genes were chosen as those with fold change > 2 and an adjusted p value for multiple testing < 0.05. The affected genes were functionally annotated, and the deregulated pathways were identified with the David database (http://david.abcc.ncifcrf.gov). The complete raw and normalized data have been deposited in the NCBI Gene Expression Omnibus (GEO) database [8] and are accessible through the GEO Series accession number GSE125498 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE125498). Venns diagrams were constructed using an interactive tool for comparing lists of genes (http://bioinfogp.cnb.csic.es/tools/venny/index.html).

Results

Patient characteristics and their peritoneal transport parameters are presented in Table 1. As expected the long-term treated patients had lower values of residual urine volume

(p< 0.01). Furthermore, long-term patients suffered more peritonitis episodes than short-term ones. No relationship between peritonitis incidence and net ultrafiltration was observed (r=-0.3, P=0.1) . However, plasma CRP and peritoneal functional tests were not different between the groups. A normal peritoneal function was defined as the presence of both D/P creatinine<0.8 and netUF>400 ml at the end of the PET.

Using microarray, gene annotation analysis of up-regulated genes in the long-term PD group showed significant enrichment of 8 genes in GO term related to cell activation (CXCR5, CD79A, CD24, TNFRSF4, LTB, HSH2D, FLT3LG, RHOH), 7 genes in GO term related to lymphocyte activation (CXCR5,CD79A, CD24, TNFRSF4, HSH2D, FLT3LG, RHOH),
7 genes related to leukocyte activation (CXCR5, CD79A, CD24, TNFRSF4, HSH2D, FLT3LG, RHOH), 10 genes associated with immune response (CCR7, POU2AF1, IL2RA, CST7, LY9, CD79A, CD24, TNFRSF4, LTB, HLA-DOB) , 5 genes related to cytokine binding (CCR7, IL2RA, CXCR5, TNFRSF25, TNFRSF4) and 7 genes involved in the cytokine-cytokine receptor interaction pathway (CCR7, IL2RA, CXCR5, TNFRSF25, TNFRSF4, LTB, FLT3LG).

To further investigate the influence of duration of PD treatment on gene activity, patients in the long-term group were divided according to peritoneal transport parameters, D/Pcreat < and ≥ 0.8 and net ultrafiltration ≤ and > 400 ml. Venn diagrams were constructed with the aim to exclude an effect of PD- associated functional alterations (Fig. 1). From 100 overlapping genes distinguishing short-and long-term patients (PD ≤ 2 years vs PD > 2 years) and concurrently patients with normal peritoneal function and an impaired PET (D/P_{creat} <0.8 vs D/P_{creat} ≥0.8 and netUF>400 ml vs netUF≤400 ml), only 22 were influenced by peritoneal function and the remaining 78 by the duration of PD treatment. Genes affected largely by peritoneal function were associated with plasma membrane signals, while the genes impacted

by PD duration were associated with immune system activation and immune response. 36 transcripts from these above mentioned 78 overlapping genes, were simultaneously increased significantly in long-term patients compared to short-term PD in the original microarray analysis (Suppl. Tab. 1). Eleven genes with the most significant difference between short- and long-term PD (CD79A, CXCR5, CCR7, CEACAM1, LTB, POU2AF1, TNFRSF25, LY9, FLT3LG, IL2RA, RHOH) were selected for validation by RT-qPCR in a larger validation cohort of 57 patients. Finally, a higher expression validated by RT-qPCR was found in LY9 (lymphocyte antigen 9) (p=0.004), CD79A (CD79a molecule, immunoglobulin-associated alpha) (p=0.032), CEACAM1 (carcinoembryonic antigen-related cell adhesion molecule 1) (p=0.032), CCR7 (chemokine (C-C motif) receptor 7) (p=0.037), and IL2RA (interleukin 2 receptor, alpha chain) (p=0.041).

To rule out the influence of cell composition of peritoneal effluent on gene activation, the proportional representation of effluent cell components was investigated. No difference in absolute counts was found between short- and long-term treated groups. No relationships between PD duration and total leukocyte count (r= -0.2; P=0.3), nor between duration of PD and absolute counts of macrophages (r= -0.2; P=0.4), and polymorphonuclears (r=-0.1, P=0.4) were observed. The peritoneal CD45+ cell population consisted primarily of monocytes (37 %). Lymphocytes comprised 22%, granulocytes 19% and NK cells 15%. T-cells accounted for 55% and B cells for 12% of the lymphocyte population. A positive relationship was present between PD duration and the proportion of granulocytes (P=0.02) and NK cells (P=0.01), but not for B-cells (Figure 2).

Discussion

In the present study the transcriptome profile in peritoneal effluent of short- and long-term PD patients was analysed. T-cell related transcripts and genes associated with cytokine activation showed increased expression in long-term PD patients. Furthermore, the number of granulocytes and NK cells increased with the time on PD treatment.

Chronic exposure to dialysis solutions during peritoneal dialysis treatment leads to persistent microinflammation of the peritoneum and subsequently activation of processes driving fibrogenesis in the long-term. Continuous exposure to bio-incompatible dialysis fluids provokes influx of inflammatory cells towards the peritoneal cavity. Alike other studies, also in the present study monocytes and lymphocytes were the most prevalent cell population in peritoneal effluent [Rodrigues-Diez *et al.* 2014; Betjes *et al.* 2015]. The majority of lymphocytes in the present study consisted of T cells. Immune status in general results from the balance between type 1 (Th1), type 2 (Th2), and type 17 (Th17) helper T-cell subsets activity. In PD patients, influx of T cells mounting a Th1-polarized response with a pro-inflammatory cytokine profile has been observed [Betjes *et al.* 2015]. The increase in the number of granulocytes and NK cells with duration of PD might reflect persistent bacterial load and infectious complications associated with PD treatment.

In our study higher expressions of *LY9* (lymphocyte antigen 9, *TNFRSF4* (tumor necrosis factor receptor superfamily member 4) and *IL2RA* (interleukin 2 receptor alpha chain) were observed in the long-term group. LY9 belongs to signalling lymphocyte activation molecule (SLAM) family receptor involved in innate and adaptive immune responses [Graham *et al.* 2006]. Assistance of *LY9* in Th2 and Th17 polarization leading to increased IL-17 secretion has been described [Graham *et al.* 2006; Chatterjee *et al. 2012*]. Intraperitoneal application of IL-17 induces an increase in expression of proinflammatory and profibrotic factors [Rodriques *et*

al. 2014]. Enhanced expression of *LY9* reflecting immune activation within the peritoneal cavity might contribute to elevated IL-17 effluent concentrations found in long-term PD patients [Rodriques *et al.* 2014]. TNFRSF4, a member of TNFR superfamily, is a costimulatory molecule for T cell proliferation. It is expressed on activated and regulatory T(Treg)cells [Klinger *et al.* 2009]. Activation of TNSFR4 by binding of his ligand launches cytokine production and promotes the differentiation of naive $CD4^+$ T cells into Th2 cells [Ohshima *et al.* 1998]. The *IL2RA* gene encodes interleukin-2 receptor alpha chain (IL-2RA, also named CD25), a component of interleukin receptor 2 (IL-2R). IL-2RA is expressed upon antigen activation mainly on resting T regulatory or activated T cells [Triplett *et al.* 2012] to bind interleukin 2 (IL2). IL2 is a pleiotropic cytokine, which modulates expression of other cytokine receptors, contributes to differentiation of naïve $CD4^+$ T cells into regulatory T cells, is involved in activation-induced cell death, in modulating T helper cell differentiation and plays an important role in the early phase of Th2 cells differentiation [Liao *et al.* 2011].

The adaptive branch of the immune system is contingent on B- and T cell activations in response to antigenic stimulation to the elimination of antigen and ensuring of lasting protection from invasion with the same pathogen. B cell activation starts with B cell antigen receptor stimulation either by antigen or by a tonic survival signal in the absence of antigen stimulation [Yasuda *et al.* 2017]. The *CD79A* gene encodes immunoglobulin-associated alpha, a part of B cell antigen receptor (BCR) [Allam *et al.* 2004]. As a higher peritonitis incidence was found the long-term group, the higher expression of *CD79A* in this patient group might reflect a prolonged pathogenic environment throughout the peritoneal cavity in the long-term PD patients, which launches the protective mechanisms enhancing an adaptive response to antigenic stimulation by B cell activation.

High glucose content and advanced glycosylation end products (AGEs) in dialysis solutions have been repeatedly shown to induce macrophage activation [Ohashi *et al.* 2010]. The M2 phenotype macrophages induced by T helper 2 cells under influence of cytokines, are involved in tissue remodeling and fibrosis via TGF beta/Smad signaling pathways [Hu *et al.* 2012]. The upregulation of CCR7 follows classicall activation of M1 phenotype macrophages by lipopolysaccharide and interferon-γ (INFγ). Activated macrophages start to produce pro-inflammatory cytokines, like INFγ and tumor necrosis factor (TNFα). These are predominantly involved in inflammation, tissue damage and killing of intracellular microbes [Mosser and Edwards 2008]. On the other hand, the CCR7 receptor and its ligands CCL19 and CCL21 control a whole range of events in adaptive immunity. Apart from other functions, CCR7 and its ligands appear to play a complex role in stimulating T cell activation and acting as co-stimulatory factors in priming T cells promoting T helper 1 and T helper 17 responses [Kuwabara *et al.* 2009]. In the present study, a higher expression of the *CCR7* gene was observed in the long-term group. An influx of macrophages towards peritoneal cavity in long-term PD patient and in an experimental model of peritoneal fibrosis has been reported [Hu *et al.* 2012]. Our data demonstrated that the proportion of monocytes increased with the time on PD. The higher expression of the *CCR7* gene might reflect M1 phenotype macrophages activation as the consequence of permanent stimulation by hyperosmolar glucose and AGEs in dialysis fluid during long-term PD treatment. Subsequently activation of T cells is induced promoting profibrotic processes.

The membrane alterations in long-term PD are accompanied with modifications of peritoneal capillaries and neoangiogenesis [Mateijsen *et al.* 1999]. Vascular endothelial growth factor (VEGF) is supposed to be involved. Kariya et al. found increased levels of VEGF-A mRNA levels in the peritoneum of patients with ultrafiltration failure, which were related to

the number of vessels and peritoneal thickness [Kariya *et al.* 2018]. In the present study a higher expression of the *CEACAM1* gene was found in the long-term group. CEACAM1 is a major effector of VEGF in the early microvessel formation, augments the effect of VEGF, and on the other hand, VEGF increases CEACAM1 expression both on the mRNA and the protein level [Ergün *et al.* 2000]. A less known function of CEACAM is its involvement in the biosynthesis of collagen. The presence of CEACAM dimers has been shown to increase the binding of the $\alpha 5\beta 1$integrin receptor to its ligand fibronectin to induce a conformational change in collagen to accelerate fibrillogenesis [Li *et al.* 2003].

The cross-sectional design is a weak point of the present study. However, a comparison of the whole transcriptome of cells in PD effluent in individual patients in relationship with PD duration and with functional changes is unique to our knowledge. In the present analysis we have demonstrated that already before the development of alterations in peritoneal function, the mechanisms of adaptive immunity promoting T helper cells response are activated in long-term PD. As adaptive immunity is antigen specific, the number of infectious complications is likely to play the role. The switch in immune response from Th1 apparent in short-term, to Th2 cells in long-term might trigger the fibrosis promoting processes.

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Tab. 1 Clinical and functional characteristic of the patients.

	Short-term PD (n=20)	**Long-term PD (n=13)**
Gender (%male)	60	70
Age at PET (years)	46(29-69)	61(33-74)[a]
Primary kidney disease (%)		
Diabetic nephropathy	10	31
Renovascular nephropathy	10	7
Glomerulonephritis	35	41
Interstitial nephritis	10	7
Polycystic kidney disease	25	7
Others and unknown	10	7
PD duration (months)	5(2-24)	43(25-68)
CRP (mg/l)	3.3(0.7-13.2)	2.2(0.2-6.3)
Renal creatinine clearance at the time of the PET (ml/min/1,73m^2)	5.1 (0-11.7)	1.0(0-9.0)
Residual diuresis (ml/24hrs)	1500(0-2500)	250(0-1750)[b]
Anuric patients at the time of PET (%)	15	61[b]
Peritoneal KT/V urea (per week)	1.5(0.6-2.3)	1.5(0.6-2.1)
Net UF at 240 min (ml)	300(-400-810)	470(-500-950)
SPT at 60 min (ml)	175(62-380)	177(36-556)
FWT at 60 min(ml)	118(68-300)	144(16-314)
D/P $_{creatinine}$	0.8(0.6-0.9)	0.8(0.6-0.9)
D/P $_{sodium}$ at 60 min	0.9(0.8-1.1)	0.9(0.8-1.0)
UF failure patients (%)	60	46
Patients without peritonitis episode (%)	50	23[b]
Patients with 1/2/3 peritonitis episodes (%)	35/10/5	23/23/31[a]

PD : peritoneal dialysis; PET: peritoneal equilibration test; CRP: C reactive protein; UF: ultrafiltration; SPT: small pore fluid transport; FWT: free water transport . UF failure was defined as net ultrafiltration of less than 400ml after a 4 hours dwell with 3,86% glucose. Values are expressed as median and ranges. [a]p<0.05 short-term v.s. long-term; [b]p<0.01 short-term v.s. long-term

Figure 1. Venn diagram showing the number of overlapping upregulated genes in long- PD patients with better functional test compared to short-PD patients with worse functional test. A segment: long-term treated patients with D/P_{creat} < 0.8 compared to short-term patients with D/P_{creat} > 0.8**; B segment:** long-term treated patients with NUF (net ultrafiltration) > 400mL compared to short-term patients with NUF≤ 400ml**; C segment:** short-term treated patients compared to long-term patients without regard to functional test results. The number in each circle (segment) represents the differentially expressed genes between the compared groups. The overlapping number stands for the mutual differentially expressed genes between the different comparisons (segments). The non-overlapping numbers represent the genes unique to each condition.

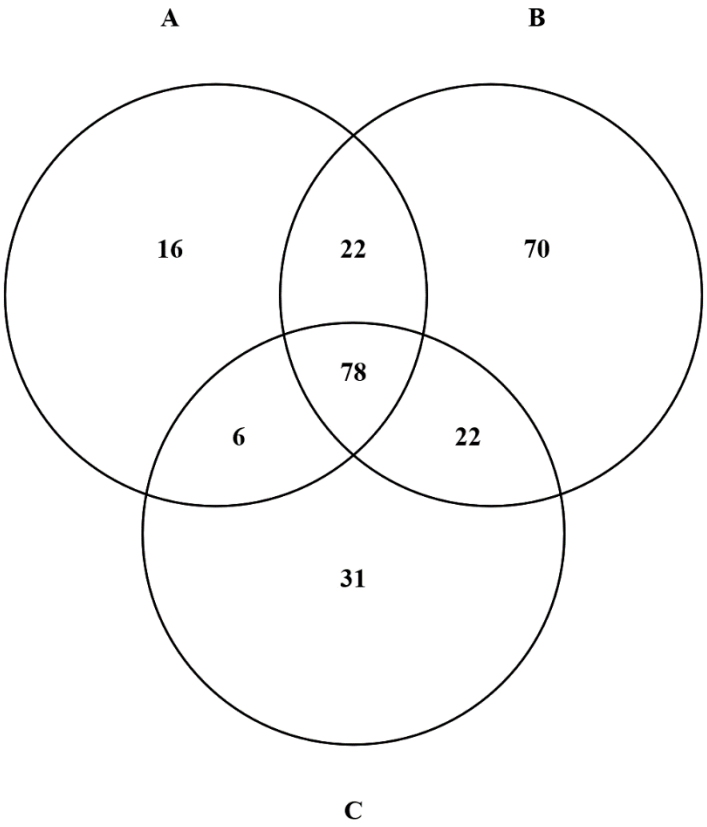
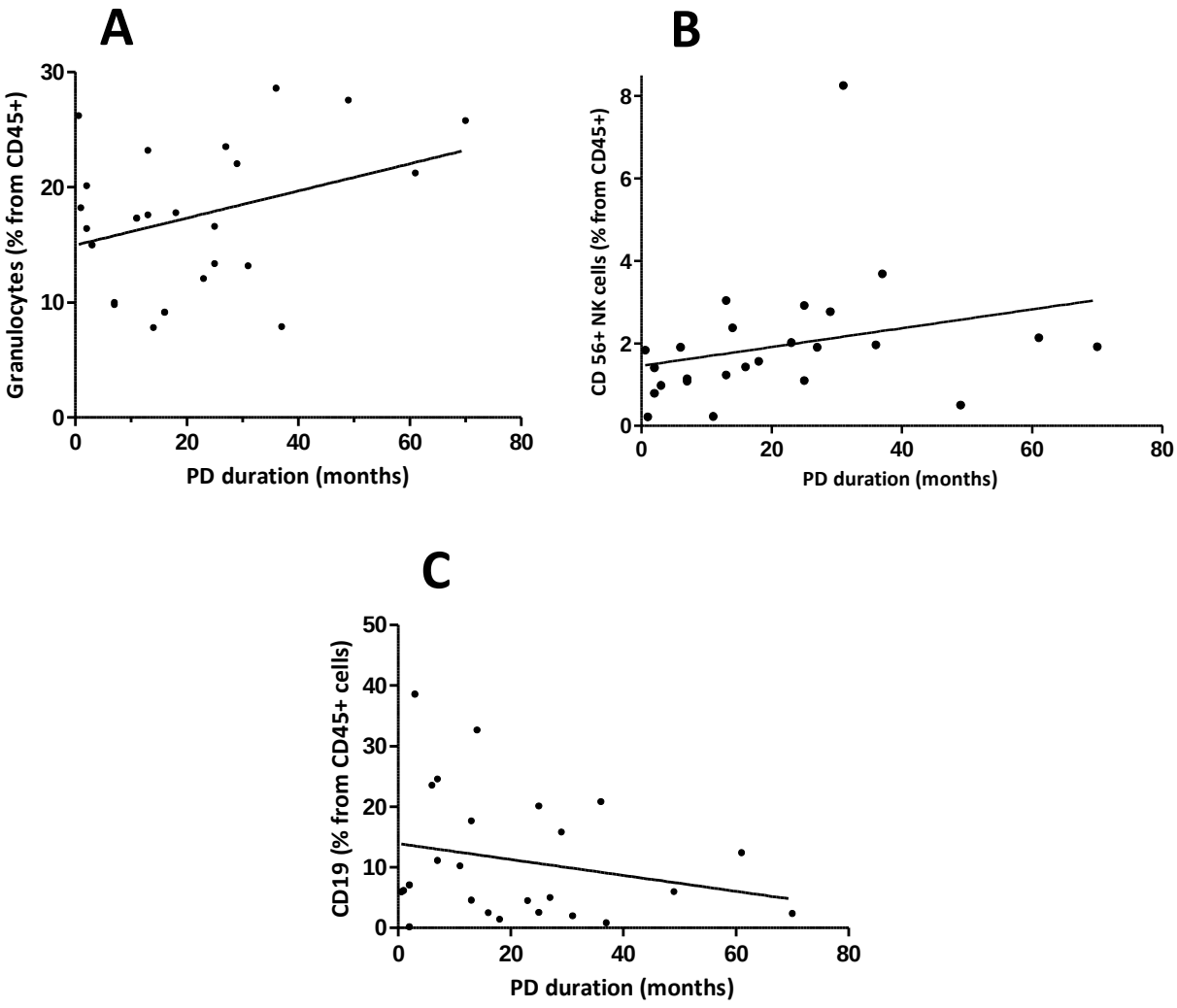


Figure 2.
A positive correlation was present between PD duration and A) percentage of granulocytes (r=0.47, P=0.02), and B) percentage of NK cells (r=0.50, P=0.01). C) The proportion of B lymphocytes (CD19 positive cells) was not related to PD duration (r=-0.22, P=0.30).



Suppl. Table 1.

The list of 36 upregulated transcripts in long-term PD compared to short-term PD patients with normal peritoneal function. *CD 79A, CXCR5, CCR7, CEACAM1, LTB, POU2AF1, TNFRSF25, LY9, FLT3LG, IL2RA, RHOH* transcripts were validated by RT-qPCR.

Symbol	Definition	FC	Adjusted P value
BLK	Homo sapiens B lymphoid tyrosine kinase (BLK), mRNA.	5.90	0.033
CD79A	Homo sapiens CD79a molecule, immunoglobulin-associated alpha (CD79A), transcript variant 1, mRNA.	5.64	0.021
CXCR5	Homo sapiens chemokine (C-X-C motif) receptor 5 (CXCR5), transcript variant 2, mRNA.	5.13	0.024
CCR7	Homo sapiens chemokine (C-C motif) receptor 7 (CCR7), mRNA.	3.78	0.033
CEACAM1	Homo sapiens carcinoembryonic antigen-related cell adhesion molecule 1 (biliary glycoprotein) (CEACAM1), transcript variant 2, mRNA.	3.73	0.020
TCL1A	Homo sapiens T-cell leukemia/lymphoma 1A (TCL1A), transcript variant 2, mRNA.	3.35	0.024
PVRIG	Homo sapiens poliovirus receptor related immunoglobulin domain containing (PVRIG), mRNA.	3.23	0.015
HSH2D	Homo sapiens hematopoietic SH2 domain containing (HSH2D), mRNA.	3.23	0.011
SOCS1	Homo sapiens suppressor of cytokine signaling 1 (SOCS1), mRNA.	3.22	0.006
LTB	Homo sapiens lymphotoxin beta (TNF superfamily, member 3) (LTB), transcript variant 1, mRNA.	3.19	0.006
POU2AF1	Homo sapiens POU class 2 associating factor 1 (POU2AF1), mRNA.	3.14	0.033
ABCB1	Homo sapiens ATP-binding cassette, sub-family B (MDR/TAP), member 1 (ABCB1), mRNA.	3.13	0.024

PTPRCAP	Homo sapiens protein tyrosine phosphatase, receptor type, C-associated protein (PTPRCAP), mRNA.	2.99	0.027
TNFRSF25	Homo sapiens tumor necrosis factor receptor superfamily, member 25 (TNFRSF25), transcript variant 10, mRNA.	2.94	0.012
LY9	Homo sapiens lymphocyte antigen 9 (LY9), transcript variant 1, mRNA.	2.92	0.006
LOC90925	Homo sapiens hypothetical protein LOC90925 (LOC90925), mRNA.	2.84	0.027
FLT3LG	Homo sapiens fms-related tyrosine kinase 3 ligand (FLT3LG), mRNA.	2.71	0.013
ZBP1	Homo sapiens Z-DNA binding protein 1 (ZBP1), mRNA.	2.54	0.025
PBX4	Homo sapiens pre-B-cell leukemia homeobox 4 (PBX4), mRNA.	2.44	0.014
HLA-DOB	Homo sapiens major histocompatibility complex, class II, DO beta (HLA-DOB), mRNA.	2.42	0.025
PPP1R16B	Homo sapiens protein phosphatase 1, regulatory (inhibitor) subunit 16B (PPP1R16B), mRNA.	2.33	0.024
CYFIP2	Homo sapiens cytoplasmic FMR1 interacting protein 2 (CYFIP2), transcript variant 1, mRNA.	2.31	0.012
LOC647450	PREDICTED: Homo sapiens similar to Ig kappa chain V-I region HK101 precursor (LOC647450), mRNA.	2.30	0.045
PLCH2	Homo sapiens phospholipase C, eta 2 (PLCH2), mRNA.	2.30	0.033
KLRB1	Homo sapiens killer cell lectin-like receptor subfamily B, member 1 (KLRB1), mRNA.	2.26	0.024
INADL	Homo sapiens InaD-like (Drosophila) (INADL), transcript variant 4, mRNA.	2.24	0.034
SP140	Homo sapiens SP140 nuclear body protein (SP140), transcript variant 1, mRNA.	2.19	0.003
LOC651309	PREDICTED: Homo sapiens hypothetical protein LOC651309 (LOC651309), mRNA.	2.17	0.012
TBC1D10C	Homo sapiens TBC1 domain family, member 10C (TBC1D10C), mRNA.	2.13	0.012

IL2RA	Homo sapiens interleukin 2 receptor, alpha (IL2RA), mRNA.	2.12	0.046
RHOH	Homo sapiens ras homolog gene family, member H (RHOH), mRNA.	2.12	0.033
ATP2A3	Homo sapiens ATPase, Ca++ transporting, ubiquitous (ATP2A3), transcript variant 2, mRNA.	2.02	0.028
CST7	Homo sapiens cystatin F (leukocystatin) (CST7), mRNA.	2.02	0.044
LOC197135	PREDICTED: Homo sapiens hypothetical LOC197135, transcript variant 5 (LOC197135), mRNA.	2.00	0.040
ISG20	Homo sapiens interferon stimulated exonuclease gene 20kDa (ISG20), mRNA.	1.97	0.041