Diploma thesis

Diagnostic benefit of effluent cytology in peritoneal dialysis associated peritonitis

A retrospective analysis of a 10 years single-center experience

written by

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Submitted in partial fulfillment of the requirement for the degree of

Doctor of Medicine

(Dr. med. univ.)

at the

Medical University of Graz

performed at the

Division of Nephrology, Department of Internal Medicine

under the supervision of

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Graz, 27.08.2019

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Danksagungen

Ganz besonderen Dank gilt Frau Dr. Anna Goritschan, die meine Arbeit betreut hat. Angefangen bei der gemeinsamen Ausarbeitung eines Themas mit interessanter These, wurde ich weiter mit unzähligen Ideen bei der Auswertung und hilfreichen Ratschlägen zur Gestaltung der Arbeit immer bestmöglich unterstützt und optimal betreut. Die konstruktive Kritik konnte mich immer wieder motivieren weiterzuarbeiten, sowie Verbesserungen durchzuführen. Somit war Frau Dr. Goritschan die treibende Kraft dieser Arbeit.
Vielen Dank für die Zeit, Unterstützung und die wirklich ausgezeichnete Betreuung.

Ebenso möchte ich mich an dieser Stelle bei meinem Betreuer Herrn Univ.-Prof. Dr. Alexander Rosenkranz bedanken, der mit zahlreichen Hinweisen und Ratschlägen verhalf, diese Arbeit weiter zu verbessern und mich während den Betreuungsgesprächen auch auf wichtige Details in wissenschaftlichen Arbeiten aufmerksam machte, die mir später sicherlich noch zugutekommen werden.
Vielen Dank für die Zeit, die Sie sich genommen haben und dass Sie mir die Möglichkeit gegeben haben, die Diplomarbeit unter Ihrer Betreuung durchzuführen.

Ebenfalls gilt auch mein Dank Frau Juliana Buchgraber, BMA, für die Bereitstellung wertvoller Informationen sowie Bilder ihrer Abteilung.

Nicht zuletzt gebührt meiner Familie, vor allem meinen Eltern, Großeltern, Tanten und meiner Schwester, sowie meinen Freunden, auf die ich mich immer verlassen konnte, mein herzlicher Dank für ihre Unterstützung.
Danke für alles. Ohne euch wäre das nicht möglich gewesen.

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List of abbreviations

ANZDATA Australian and New Zealand Dialysis and Transplant Registry

APD Automated Peritoneal Dialysis

BMI Body Mass Index

CAPD Continuous Ambulatory Peritoneal Dialysis

CCPD Continuous Cycling Peritoneal Dialysis

CKD Chronic Kidney Disease

CNP Culture-negative Peritonitis

CNS Coagulase-negative Staphylococci

CPP Culture-positive Peritonitis

CNP Culture-negative Peritonitis

DM Diabetes mellitus

E. coli Escherichia coli

EP Eosinophilic Peritonitis

ESI Exit-side-infection

HD Hemodialysis

IE International units

Ig Immunoglobulin

IPP Intraperitoneal pressure

IPV Intraperitoneal volume

ISPD International Society for Peritoneal Dialysis

LAL Limulus amebocyte Lysate

MEDOCS Steiermärkisches medizinisch-pflegerisches Dokumentations- und Kommunikationsnetzwerk

MRSA Methicillin-resistant Staphylococcus Aureus

NF-κB Nuclear Factor 'kappa-light-chain-enhancer' of activated B-cells

NIPD Nocturnal Intermittent Peritoneal Dialysis

OEDTR Österreichisches Dialyse- und Transplantationsregister

P. aeruginosa Pseudomonas aeruginosa

PD Peritoneal Dialysis

S. aureus Staphylococcus Aureus

S. epidermis Staphylococcus Epidermis

SLE Systemic Lupus Erythematosus

STWD Sterile Tubing Welder Device

TNF Tumor necrosis factor

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Zusammenfassung

Einleitung

Peritonitis, als Komplikation der Peritonealdialyse (PD), gilt als der führende Umstand für Behandlungsinsuffizienz und Mortalität. Für eine zielgerichtete Antibiose ist eine mikrobielle Diagnostik obligatorisch, welche mittels Kultur des Auslaufdialysats durchgeführt wird. Da diese bis zu mehreren Tagen in Anspruch nehmen kann und auch Kultur-negative Ergebnisse auftreten, könnte eine mikrobiologische Analyse Abhilfe schaffen und diese Schwächen kompensieren. Diese Arbeit versucht zu evaluieren ob eine solche Analyse zusätzliche Vorteile in der Diagnose einer Peritonealdialyse-assoziierten Peritonitis erbringt.

Methoden

In dieser retrospektiven Einzelzentrumsstudie wurden alle PD Patienten eingeschlossen, die zwischen 01.01.2007 und 31.12.2017 am Univ.-Klinikum Graz betreut wurden. Die mikrobiologische Untersuchung, bestehend aus einer automatisierten Zellzählung, einer Hemacolor-Schnellfärbung, Übersichtsmikroskopie und Gramfärbung, wurde bezüglich des Nutzens evaluiert und der Auslauf-Kultur gegenübergestellt.

Ergebnisse

Von 253 PatientInnen, die in der PD-Ambulanz betreut wurden, wurden in diese Studie 250 PD-PatientInnen (68% männlich, 56±15 Jahre bei PD Start) miteinbezogen. Es traten 155 Peritonitisepisoden in 662,7 Jahren unter Risiko auf (0,234 E/a). Eine Gramfärbung konnte in 52% der Kultur-negativen Peritonitiden (CNP), aber nur in 63% der Kultur-positiven Peritonitiden (CPP) einen Erreger identifizieren. Zehn von 41 CNP zeigten hauptsächlich eosinophile Granulozyten im Dialysatauslauf. Eosinophile Episoden präsentierten sich initial mit 568 Leukozyten/µl deutlich niedriger als neutrophile CNP (1820/µl; p=0,199) und CPP (8346/µl; p<0,001) und mussten seltener zur Hämodialyse wechseln (0%), verglichen mit neutrophilen CNP (16.1%) und CPP (15.7%). CNP mit positivem Ergebnis in der Gram-Zytologie, im Vergleich zu CNP mit negativem Befund, zeigten einen Trend zu initial höheren Leukozyten-Werten (2082/µl vs. 1541/µl, p=0,892), einer längeren Zeit bis die Leukozyten wieder <100/µl waren (7,92 Tage vs. 7,15, p=0,545) und häufigeren Transfers zur HD (18,7% vs. 13,3%, p=0,686), wenn auch statistisch nicht signifikant.

Zusammenfassung

Die in den Guidelines empfohlene Mikroskopie des Gram-gefärbten Dialysatauslaufs kann additiv zur Diagnosestellung und Keimidentifizierung einer PD-assoziierten Peritonitis

eingesetzt werden. Die zentrumspezifische standardisierte Färbe-Prozedur erbrachte in mehr als der Hälfte der CNP einen Keimnachweis und dient somit als Wegweiser zur zielgerichteten Therapie. Die Mikroskopie des Hemacolor-gefärbten Dialysatauslaufs konnte zwischen eosinophilen und neutrophilen Peritonitisepisoden differenzieren, die in ihren Charakteristika deutlich voneinander abweichen und liefert somit nicht nur anamnestisch-diagnostische Informationen, sondern suggeriert auch eine spezifische Therapie. Weitere Studien werden empfohlen.

Abstract

Introduction

Peritonitis, as complication of peritoneal dialysis (PD), is the major cause of treatment failure and deaths. For targeted antibiosis, microbiological diagnosis, performed via cultures, is obligatory. Nevertheless, this workup takes time and culture-negative results appear occasionally. Therefore, microscopical analysis might be a useful tool to compensate those weaknesses. This study tries to evaluate if this method has additional benefits in diagnosis of peritoneal dialysis associated peritonitis.

Methods

We performed a single-center, retrospective analysis, including all PD patients, taken care of between 01.01.2007 and 31.12.2017 at the university hospital in Graz. Microbiological examination, consisting of automated quantitative cell count, Hemacolour stain, microscopic sediment survey and Gram stain, was evaluated regarding benefits and compared to effluent culture.

Results

Of 253 patients taken care of at the PD department, 250 patients (68% male, mean age 56±15 years at PD start) performing PD-only were included. In 662.7 years at risk, 155 episodes of peritonitis occurred (0.234 episodes per patient year). Gram stain detected in 52% a possible germ in culture-negative peritonitis (CNP), but only in 63% of culture-positive episodes (CPP). Ten episodes out of 41 CNP primarily showed eosinophilic granulocytes, with different characteristics regarding initial effluent leucocytes (568/µl), compared to neutrophilic CNP (1820/µl; p=0.199) and CPP episodes (8346/µl; p<0.001) and switch to hemodialysis (0%), compared to neutrophilic CNP (16.1%) and CPP (15.7%). CNP with positive results in Gram's cytology, compared to CNP with negative result, showed a trend to higher initial leucocytes (2082/µl vs 1541/µl, p=0.892), longer time until leucocytes were below 100/µl (7.92 vs. 7.15 days, p=0.545) and more frequent switch to HD (18.7% vs 13.3%, p=0.686), although statistically not significant.

Conclusion

Microscopical analysis of Gram's stain, recommended by the guidelines, can be used additionally for diagnosis and germ identification in peritoneal dialysis associated peritonitis. The centrum-specific standardized colour method detected in more than a half of CNP a germ und can be used as a guide to targeted therapy. Microscopy of the Hemacolor stain distinguished between eosinophilic and neutrophilic episodes, which

differ in their characteristics, and therefore not only gives anamnestic-diagnostic information, but also suggests further treatment. Further studies are recommended.

1 Introduction

1.1 Peritoneal Dialysis

Peritoneal Dialysis (PD) is one of the options for kidney replacement therapy in patients with end stage renal failure, besides hemodialysis or kidney transplantation. Installing a peritoneal dialysis solution into the peritoneal cavity, the peritoneum is thereby used as a semipermeable membrane, which features the physical principles of diffusion, ultrafiltration, osmosis and convection to enable solutes and water cross from the peritoneal capillaries to the dialysate solution. The dialysis solution is exchanged via an abdominal catheter in the abdominal cavity several times daily. The peritoneal membrane with a total exchange surface of 1-2 m^2 is subdivided anatomically into the parietal and visceral peritoneum and consists of a monolayer of mesothelial cells, stocked with microvilli to enhance the surface of interchange, followed by the interstitial space that contains not only collagen fibres, but also the peritoneal capillaries (1).

1.1.1 Types of peritoneal dialysis

There are different variants of performing PD in order to have an adequate elimination of excess water and substances such as urea, creatinine, potassium and many more. Both variants are based on the same principle, whereby a special solution, the peritoneal dialysis solution, is filled in the peritoneal cavity (dialysate influent), remains a certain time in the peritoneal cavity (dwelling time) and is then drained into an empty bag in order to empty the cavity (dialysate effluent).

1.1.1.1 Continuous ambulatory peritoneal dialysis

Firstly, there is the continuous ambulatory peritoneal dialysis (CAPD). The patient manually exchanges the fluids by himself, using the gravitational force to fill and drain the dialysate solution into and from the peritoneal cavity. This technique might be appropriate for patients with a distinct daily routine since the exchanges are performed (about every 4 hours) during daytime. Usually, no exchanges are necessary during the night.

1.1.1.2 Automated peritoneal dialysis

On the other hand, there is the alternative to use machines that perform the exchanges automatically. Therefore, this method is called automated peritoneal dialysis (APD), in which so-called “cyclers” perform multiple exchanges mostly during the night, while patients are sleeping and as a consequence thereof, provide the advantage of a dialysis-free

daytime for most of the patients. Depending on the filling volume during daytime, which can be installed by the cycler in the morning and therefore dwells during the whole daytime, the continuous cycling peritoneal dialysis (CCPD) must be distinguished from the nocturnal intermittent peritoneal dialysis (NIPD). While during the CCPD a certain dwell volume is kept inside the cavity during the day to enhance the transposition of solutes, the NIPD is characterized by a dry peritoneal cavity throughout the day.
In certain cases, in need of more intense dialysis quality or ultrafiltration, methods can be combined, as patients undergoing CCPD use additional manually exchanges during daytime.

1.1.2 Indications for peritoneal dialysis

Peritoneal Dialysis is an ideal treatment for patients who either do not tolerate hemodialysis, mainly due to symptomatic arterial hypotension, or are compromised as a result of vascular failure. Other situations, such as living far away from a hemodialysis unit, age between 6 and 16 years, active lifestyle, bleeding diatheses, multiple myeloma and chronic infections are mentioned reasons to prefer peritoneal dialysis as treatment (2). In cases of cardiorenal syndromes, solely drainages of cardiogenic ascites often improve or stabilize kidney function; therefore, PD start can be delayed and the catheter, moreover, gives the opportunity to easily manage intermittent dialysis, due to recurring decompensation of heart failure with therapy refractory hypervolemia. In a recently conducted study in patients with cardiorenal syndrome, PD positively affected the blood urea nitrogen/creatinine ratio (148.7 ± 68.3 to 106.7 ± 44.8 mg/dL, p= <0.001) and hospitalization rates (39.2 ± 30.7 to 27.1 ± 25.2 days, p=0.004), as well as New York Heart Association functional class (3.38 ± 0.55 to 2.85 ± 0.49, p= < 0.001) (3). Also, venous congestion and right ventricular systolic pressure is reduced in patients with cardiorenal syndrome, treated with PD (4). In this kind of patient population, PD might be the preferable type of kidney replacement therapy.

1.1.3 Complications

Because of the completely different method for ultrafiltration and clearance, compared to hemodialysis, and therefore other accesses and special dialysis solutions, different, design related problems occur.
Regarding peritoneal dialysis, the following complications are well documented: Hernias, abdominal wall or pericatheter leaks, genital edema, respiratory complications such as hydrothorax and altered mechanics of breathing, back pain, overfill, tunnel-infections,

dislocation of the catheter, exit-site-infections, peritonitis and encapsulating sclerosis are known as adverse events (5). Furthermore, also dyspepsia and the presence of gastroesophageal reflux symptoms have been described as complications (6).
Hernias are estimated to occur in 10-20% of patients, but the exact number is not easy to define, since lots of hernias are asymptomatic (5). Also, abdominal wall and pericatheter leaks are quite common, especially as a postoperative complication. Both are mainly due to large volumes, instilled into the abdominal cavity additional to other factors which increase the intraperitoneal pressure (5). Other non-infectious complications owed to higher intraabdominal pressure are hydrothorax, overfill gastroesophageal reflux or back pain.
While exit-side infections and tunnel infections are primarily caused by direct inflammation with germs of the healthy human skin flora such as Staphylococcus Aureus (S. aureus), Pseudomonas aeruginosa (P. aeruginosa) or Staphylococcus epidermidis (S. epidermidis), occurring once every 24-48 patient months (7), the exact pathogenesis of peritonitis is still a matter of debate.

1.1.3.1 Peritoneal dialysis associated peritonitis

Although the number of infectious complications decreased over the past decades, as a result of advanced dialysis techniques, prophylactic use of anti-infectious medication and improving training techniques for handling peritoneal dialysis, about 30% of treatment failure and 16% of deaths among patients on PD are still caused by the occurrence of a peritonitis, happening approximately once every 20-60 patient months (8). Less than 5% of peritonitis lead to death, but about 16% (9) to 20.8% (10) have to switch either temporarily or permanently to hemodialysis (HD) due to this complication and subsequently PD technique failure, respectively membrane failure (11). According to a study, using the ANZDATA register, after transfer to HD, only 18% could return to PD (9). In another published research, conducted in Australia and New Zealand observing 4675 patients between 2003 and 2006 (12), culture-negative peritonitis, compared to culture-positive peritonitis, is less likely associated with transfer to haemodialysis (10% vs 19%; $P < 0.01$), death (1% vs 2.5%; $P < 0.04$), hospitalization (60% vs 71%; $P < 0.01$) or catheter removal (12% vs 23%; $P < 0.01$) and has therefore a more benign outcome.

There are different pathogenetic pathways of infections. First of all, because of handling failure/issues occurring while connecting the transfer set, bacteria such as S. aureus or P. aeruginosa, gain access to the cavity. These bacteria are known to produce a biofilm on foreign surfaces and are therefore of major concern for chronic infections (13).

In a recent study, observing 4675 PD-patients, 503 of 3594 episodes of peritonitis were caused by S. aureus and infections caused by this germ showed more often relapses of peritonitis compared to non-S. aureus peritonitis (20% vs 13%) as well as slightly elevated numbers of catheter removals (23% vs 21%)(14). The presence of methicillin-resistant staphylococcus aureus (MRSA) seems to be predictive for permanent hemodialysis transfer (14) and Pseudomonas spp. are associated with adverse outcome (15).
Otherwise, there is also the possibility for bacteria to migrate from the intestinal lumen of the bowel through the bowel wall into the cavity and are therefore called “enteric peritonitis”. Bacteria involved in this kind of pathway are typically Escherichia Coli (E. coli) and Klebsiella sp. (8). Also, but less often, there were hematogenous and transvaginal ways observed, involving E. coli, Enterococcus faecalis, Enterobacter spp. et cetera (8).
Another issue seems to be the role of host defenses. The leukocytes, which cope with invading bacteria, are depending on the milieu and therefore on the pH, osmolality and the presence of lactate anions. Low pH and high osmolality in the peritoneal cavity, which are common characteristics of the most PD solutions, are blocking the mechanisms of the leucocytes (8). In contrast, oral vitamin D treatment is associated with lower rates of peritonitis (16), while vitamin D deficiency is associated with greater likelihood of peritonitis (17). Bacchetta et al. showed in a study, performed in 2014, that vitamin D supplementation promotes innate immune responses that possibly supports macrophage antibacterial response in PD-patients (18).

1.1.3.1.1 Diagnosis of peritoneal dialysis associated peritonitis

According to the guidelines of the International Society for Peritoneal Dialysis (ISPD), the diagnosis of a peritonitis is based on three columns. At least 2 of the following 3 attributes have to be present to diagnose a peritonitis (8).
-Symptoms: Common symptoms including abdominal pain, vomiting and nausea. These symptoms might be missing in elderly sometimes, therefore rapidly decreasing residual renal function and postural hypotension are the only evidence for peritonitis (8).
-Cloudy effluent: The effluent liquid turns foggy, when there are more than 50-100 cells/µL. For diagnosis of this infection, the dialysate leucocytes have to exceed 100/µL after 2 hours of dwell time, of which more than 50% have to be polymorphous. A cloudy effluent fluid can also be caused by other reasons, like presence of blood, fibrin, chylus. It can even be discolored by special drugs, such as calcium channel blockers like lercanidipine (19, 20), resulting in the importance of a differential cell count with, typically, more than 50% polymorphous neutrophil granulocytes (8).

-Verification of bacteria in the dialysate performed by Gram's stain or culture.

1.1.3.1.2 Identification of Causative Organism

The identification of the causative organism is not only important in order to choose the right antibiotics, but also, to receive relevant information about the source of infection and therefore enabling physicians and patients to take precautions to prevent recurrent, relapsing or repeat of peritonitis. By inoculating 5-10 mL of effluent into both, aerobic and anaerobic, blood-culture bottle kits bedside, followed by centrifuging and culturing the pellet, culture-negative rate is typically 10 to 20% (21, 22). The ISPD Guidelines recommend using rapid blood-culture bottle kits rather than standard blood-culture bottles to optimize culture rate.

Alternative methods, such as drawing 50 ml effluent, centrifugate it at 3.000 g for 15 minutes, then resuspend the sediment in 3-5mL supernatant and inoculate on solid culture media or standard blood culture media, increase the culture rate up to 5 to 10 times (23, 24). Compared to water lysis method, the preliminary organism identification rate by Gram's staining with the broth inoculation culture is better (70.6% vs 17.6%), especially when gram-positive pathogens were involved, and also faster (1.3 ± 0.7 vs. 2.6 ± 1.6 days)(24). Other substances such as Tween 80, which is a non-ionic emulsifier, are enhancing the biofilm growth of S. aureus and could be therefore used to optimize the diagnostic sensitivity (25).

The sample should be delivered to the laboratory within 6 hours and incubated in aerobic, microaerophilic and anaerobic environment. If instant consignment is not possible, the sample should be incubated at 37 degrees. Normally, the diagnosis should be determined within 3 days. If this is not the case, the effluent sample should be analyzed further with repeat cell count, differential count and fungal and mycobacterial culture shall be conducted. Furthermore, the culture media should be incubated 3 to 4 more days to detect slow growing bacteria (11).

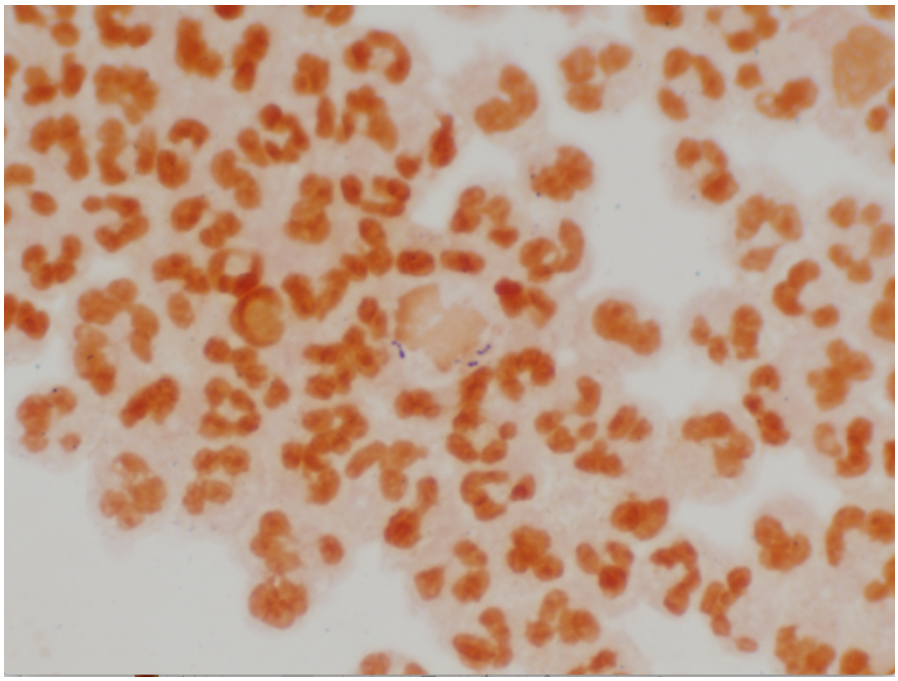

Figure 1: Gram-positive streptococci (Source: Juliana Buchgraber, Medical University of Graz, Division of Nephrology, Department of Internal Medicine)

In Figure 1, gram-positive streptococci can be spotted between granulocytes in peritoneal dialysis effluent cytology, as observed in this case of streptococcus associated peritonitis.

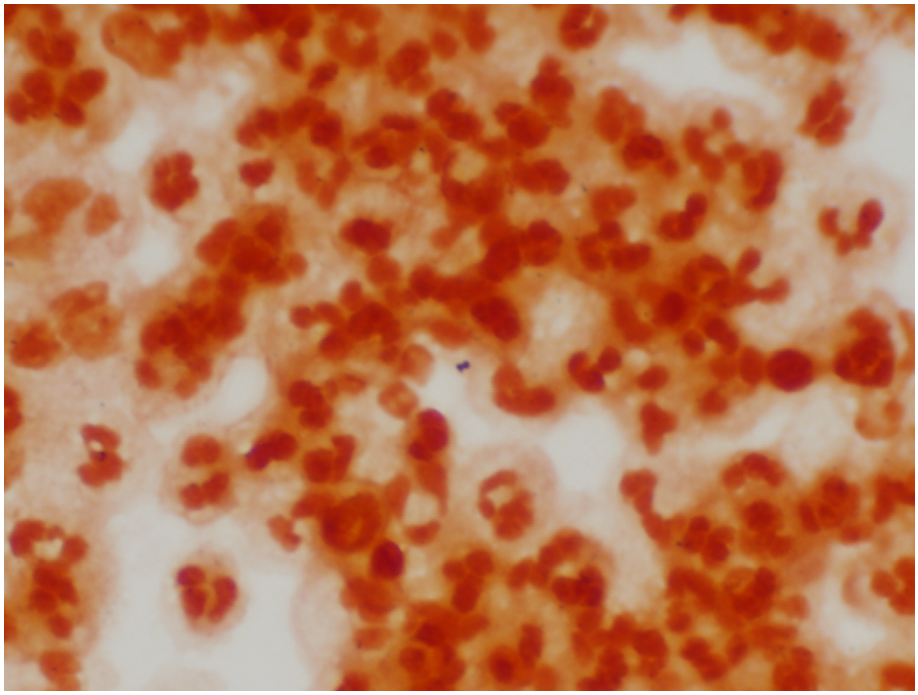

Figure 2: Gram-positive cocci (Source: Juliana Buchgraber, Medical University of Graz, Division of Nephrology, Department of Internal Medicine)

In Figure 2, dialysate effluent cytology with gram-positive cocci between granulocytes is pictured.

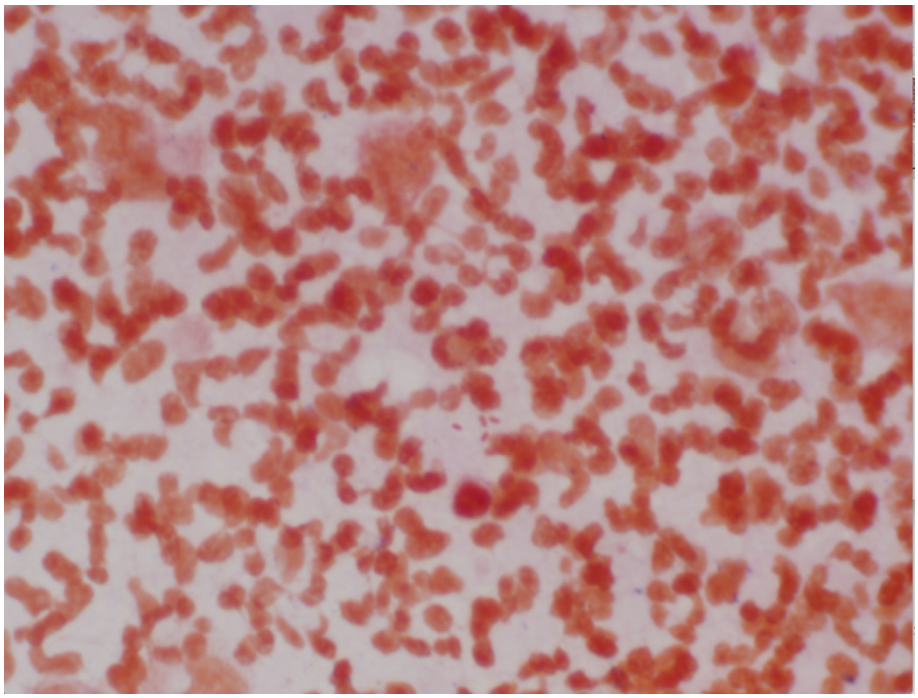

Figure 3: Gram-negative rods (Source: Juliana Buchgraber, Medical University of Graz, Division of Nephrology, Department of Internal Medicine)

Dialysate effluent cytology with gram-negative rods between granulocytes is shown in Figure 3.

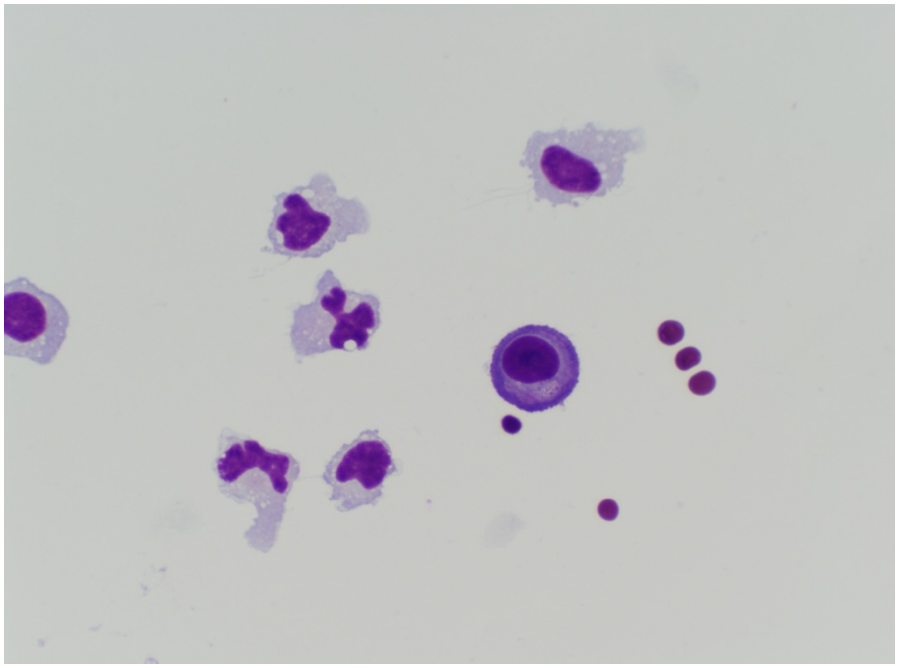

Figure 4: Lymphocyte and reactive mesothelium (Source: Juliana Buchgraber, Medical University of Graz, Division of Nephrology, Department of Internal Medicine)

Reactive mesothelium, as pictured in Figure 4, may be seen in microscopy during an episode of peritonitis and may give a lead to the medical condition. Nevertheless, it is not considered as one of the 3 criteria of diagnosis of a peritoneal dialysis associated peritonitis.

1.1.3.1.3 Terminology of peritoneal dialysis associated peritonitis

In terms of terminology of peritonitis, there are different types to distinguish (11).
-Recurrent peritonitis occurs when an episode appears within 4 weeks of completion of therapy of a prior episode, but with a different organism.
-A relapsing peritonitis is defined as occurrence of a peritonitis episode within 4 weeks of completion of therapy after a prior episode with the same organism or one sterile episode.
-A repeat of a peritonitis takes effect when an episode with the same organism occurs more than 4 weeks after the therapy was completed.

While relapsing episodes should not be counted as another episode, repeats and recurrent are supposed to be counted for calculation of peritonitis rate.

1.1.3.1.4 Calculation of peritonitis rate

There are different ways to calculate the accurate peritonitis rate (26).
- Centre-referential calculation of peritonitis rate as episodes per patient year:
Total number of PD patients' days at risk/365 days per year = patient years of experience
Number of episodes of peritonitis of all patients / number of years of experience of all patients = episodes per patient year
- Centre-referential calculation of peritonitis rate as percentage of patients per period of time who are peritonitis free.
- Patient specific calculation of peritonitis rate as 1 episode per number of patient months:
Total number of PD patient days at risk / 30.4 days per month = patient months experience
Number of patient months of experience / number of episodes of peritonitis = 1 episode per number of patient months.

1.1.3.1.5 Risk factors for peritoneal dialysis associated peritonitis

Numerous risk factors for peritoneal dialysis associated peritonitis have been described.

Comorbidities related factors:

Diabetes mellitus (DM): In 2013, a multi-center study in Turkey was published, which observed 915 patients between 2000 and 2010, including 217 PD patients with diabetes

mellitus (27). The aim of this study was to define predictors of mortality and morbidity between diabetic and non-diabetic PD patients. They did not only detect that the mortality in the diabetes group was significantly higher, but also the risk for peritonitis increased with this comorbidity. This finding is supported by several studies (28-34) while others (35-38) did not find this association, e.g. Lobo et al. (37) who did only find a correlation among women with diabetes mellitus. Furthermore, diabetes mellitus seems to be an independent risk factor for intestinal bacterial overgrowth, which seems to affect the level of potassium (39).

Underlying renal disease: The underlying renal disease, such as Systemic Lupus erythematosus (SLE), seems to be a predisposing factor for infections and worse technique survival. In patients with SLE, this might be due the use of steroids, since the use of these drugs is associated with a higher incidence of peritonitis ($p = 0.04$), but also because systemic lupus erythematosus itself may further compromise the immunity of uremic patients (40). Collagenosis, with that SLE, is also associated with a negative predictive value for outcomes of PD-related peritonitis, due to the negative impact of treatment with vancomycin (15).

Nutritional status and hypoalbuminemia: Hypoalbuminaemia is a well described risk factor for peritonitis in PD patients (16, 28, 35, 41-48) which also favors adverse outcome (49). A subsequently increased inflammatory response and malnutrition paired with non-adequate immune responses is thought to be the explanation to higher rates of peritonitis (45). In a cohort study, consisting of 305 PD patients, a daily protein intake of 0.94 g/kg/day was linked to significantly higher serum albumin level and subsequently less first episodes of peritonitis, compared to patients with a daily protein intake, less than 0.73 g/kg/day (50).

Other comorbidities: McDonald et al. (30) observed an elevated risk for peritonitis related to comorbidities such as coronary artery disease and chronic lung disease, while Oo et al. (29) found a relation between congestive heart failure and peritonitis. After adjusting for PD modality, age, sex, race, primary cause for end-stage-renal-disease, the number of entry-period hospital days, peritonitis during the entry period and entry hematocrit value, the average number of months until first episode of peritonitis, 9 months after PD-start has been 16.0 months with congestive heart failure and 17.8 months without congestive heart failure (29).

PD- technique related risk factors:

Catheter design: There are different designs of PD catheters.

First of all, there is the standard or straight connection system, whereby the dialysate solution bag is connected to the catheter via a tubing and a ‘spike’ or ‘luer lock’ system (61). At the influent stream of dialysate, a new bag is used and afterwards it is remaining rolled up for the effluent dialysate.

Moreover, there is the Y-set, whereas the patient disconnects during the exchanges. In order to drain dialysate with the Y-set system, one limb is connected to an empty drain bag and the other one to a new bag with fresh dialysate. Another advantage of the Y-set is the flush before fill technique, whereby air can be safely released into the drain bag.

The double bag system was a further development of the Y-set system, whereby the patient has to perform one less connection due to the corresponding bags (61).

Nishina et al. (33) found that the use of a sterile tubing welder device (STWD) as a connection system is associated to higher risks of peritonitis. In a systematic review of 2001 including 991 CAPD patients, twin-bag and y-set were compared with the STWD (51). Out of 363 patients, who used the y-set or double-bag system, 133 (37%) developed an episode of peritonitis while patients, who stuck to STWD design, developed a peritonitis in 158 out of 263 cases (60%) (OR 0.33). When comparing the double bag system with the y-set, the double bag was found to be superior (51).

In another systematic review, Y-Set and twin-bag, which is sort of a development of the Y-set, whereby the connection to the fresh bag is already made, were the only systems, which were found to be effective in preventing PD-related peritonitis (52). Using these systems was a major improvement in order to prevent peritonitis, mainly due to less connections, which have to be performed and also because of establishing the “flush before fill” technique, which contributes to the fact that less air gains access to the abdominal cavity. Interestingly, the ISPD Guidelines have no recommendation for a specific catheter design in order to prevent peritonitis (11).

Technique of catheter placement: A recent meta-analysis performed in 2015, comparing thirteen studies with a total of 2681 patients, concluded that there is no difference in 1-year survival in percutaneous versus surgical placement but reported about a lower occurrence of peritonitis in patients with percutaneous placement (RR 0.77) (53).

Exit-Site-Infection (ESI): In a Canadian cohort study, which included 962 PD patients, it was shown that the risk for peritonitis after an ESI, in spite of appropriate antibiotic treatment, was higher for all gram-positive infections, including coagulase-negative staphylococci (CNS) and S. aureus, but not for culture-negative and gram-negative germ infections (54). Also, the time to subsequent peritonitis, was shorter after at least one ESI,

compared to patients with no ESI ($p<0.001$) (54). Wu et al. and van Diepen et al. (43, 55) came to a similar result as well as Boehm et al. (56) in pediatric peritoneal dialysis patients. Kofteridis et al. (57) showed that ESI affect the outcome of PD-related peritonitis.

PD Duration: There are contrary statements considering the length of peritoneal dialysis therapy on how it affects the appearance of peritonitis. Two studies (49, 58) found a coherence whereas others (57, 59, 60) did not. While the study group of Perez Fontan et al. found that time on PD at the time of the event was a predictor of mortality (OR 1.02/month), Krishnan et al. reported that the nonresolution rate of patients who had been on PD for more than 2.4 years was 24,4% and the rate for those under 2.4 years of treatment had been 16.5% (49, 58).

PD Modality: Studies covering the incidents of peritonitis regarding the used PD-modality are varying. Whereas two studies (29, 38) found that APD is related to higher risk of peritonitis compared to CAPD, other studies (61-63) came to the opposite result. A study published in 2014 by Lan et al., including 6959 Australian PD patients, who were treated with PD between 2003 and 2011, demonstrated that there is no difference between both modalities regarding the risk of peritonitis which may be attributable to the fact that the other studies were performed in the past millennium and had a lower number of included patients (64). This finding of Lan et al. is coherent with other studies (37, 65-69).

Miscellaneous risk factors:

Age: In a single center, 10-year retrospective study on risk factors for peritoneal dialysis-related peritonitis at Tokai University Hospital, researchers found that higher age at the start of PD is an important risk factor (33).

Further on, there is evidence that also the age, at which PD is performed, influences the risk of peritonitis (10, 36, 42, 70-76) and favors adverse outcome (32, 49). A possible explanation is the decreased dexterity and vision in elderly patients (42) or the coherence between morbidity and older age (77). Martin et al. (78) did not find an association between age and incidence of peritonitis.

In contrast to those conclusions, Nessim et al. (79) collected data from 1996 to 2005 of multiple study centers, which included 4247 patients treated with PD and defined two eras of PD initiation (1996-2000 and 2001-2005), because the year-by-year analysis showed a decreasing significance for the correlation of age and peritonitis after the year 2000. They found that, although increasing age was associated with a higher occurrence of peritonitis overall, this did not coincide with patients in the second era of PD initiation. They implied that age is more an era effect as a risk factor per se and tried to explain the different

outcomes of the studies mentioned above, through that they had a limited statistical power, due to small study size, a different cut-off age, which defined the word “elderly”, and the time period in which the studies were performed since, some of these were performed in the late 1980s. In summary, after the year 2001, there is no association between higher occurrence of peritonitis and increasing age (79). A similar effect was reported by McDonald et al. (30), describing lower peritonitis rates in the last couple of years as “vintage effect”. This is in accordance with a more recent study published in 2016 by Duquennoy et al. including 8396 patients with 3173 patients aged older than 75, who found that risk of peritonitis in elderly patients is not increased (80).

Body Mass Index (BMI), intraperitoneal volumes and intraperitoneal pressure: In 2004, a retrospective cohort study among 10709 Australian and New Zealand PD patients, was published, which observed patients between 1991 and 2003. They revealed, that higher BMI leads to a first peritonitis episode at a faster pace (30).

In a prospective observational study, published by Prasad et al. (81) in 2014, including 365 patients with end-stage renal disease, they discovered that the OR, compared to normal-weight patients for developing a peritonitis, is 1.7 in overweight and 3.4 in obese patients. Also, episodes of peritonitis were more frequent in obese patients than in normal-weight patients (81). In another retrospective cohort study by Wu et al. (43), higher BMI led to more early-onset peritonitis. In 2007, a study was published, which revealed, that the intraperitoneal pressure (IPP) after installing the same intraperitoneal volume (IPV) was significantly higher in patients with higher BMI and showed a linear correlation between IPV and IPP (82). Patients with IPP over 14cmH_20 during the night, are more likely to have a peritonitis episode. A similar, but not significant, effect of pressure was observed during daytime. This is in agreement with Scanziani et al. (83). In addition, for 3 days after catheter implantation, the IPP is found to be higher, regardless which intraperitoneal volume is instilled (84). There also seems to be a coherence of higher intraperitoneal volume to the appearance of proinflammatory cytokines such as tumor necrosis factor (TNF) alpha and interleukin-6 (85).

Constipation: While Mishalov et al. (44) reported that constipation is a risk factor for peritonitis, Singharetnam and Holley (86) came to the result that acute treatment of constipation is also related to the occurrence of peritonitis. The ISPD Guidelines suggest proper treatment of problems like constipation or gastroenteritis, which are common during PD therapy, although there is no evidence that treatment reduces the rate of peritonitis (11).

Gender: The literature reporting if gender is associated with increased risk for peritonitis is inconsistent. Kotsanas et al. (70) did find a coherence between increased rates of peritonitis and female gender, observing a cohort of 506 patients (OR 1.91) while Fan et al. (42) reported that rather male gender is related to higher risk for a first episode of peritonitis (HR 1.315), examining 1117 patients performing CAPD. Observing 565 patients, Perez Fontan et al. (49) reported that mortality due to peritonitis was higher in females (RR 2.13), possibly caused by different genitourinary tract among women since it may happen to be a reservoir of micro-organisms. A recent study in 2014, observing 1378 patients, reported lower risk for females (RR 0.85) (87). At the moment it can be stated that the actual situation of studies is inconclusive.

Immunocompromised Condition: In 1996, Andrews et al. (88) observed elevated rates of CAPD peritonitis among patients with current immunosuppressive therapy as well as a quite recently immunosuppression and showed a relationship between the aggressiveness of immunosuppression and the incidence of peritonitis and reported furthermore that 5 of 6 cases of immunosuppressed patients with fungal peritonitis received a combination of cyclophosphamide and methylprednisone. Immunosuppressed patients, due to morbidities such as SLE and previous transplantations (88) are therefore considered as risk factors for peritonitis (40, 89).

Prior Peritonitis: Golper et al. (38) found an association between previous episodes of peritonitis and increased risk for further peritonitis.

Prior renal placement therapy: Several studies (37, 89, 90) found that prior hemodialysis is a risk factor for peritonitis (37, 90) and early PD failure (89), maybe due to suppressed immune system and consequent raised risk of bacteremia. Bechade et al. concluded that failed transplant patients are also at higher risk for PD failure, maybe due to continued immunosuppressive therapy (89).

1.1.3.1.6 Causes of culture negative peritonitis

Culture-negative peritonitis may be due to infectious reasons such as prior treatment with antibiotics (12, 91, 92). Also, technical problems during the effluent dialysate culture have been described as reasons for undetectable organisms (91, 92). On the other hand, there are also noninfectious triggers, such as chemical irritation caused by the catheter or the dialysate (92).

When more than 10% of eosinophil amounts to the total white blood cells in the dialysate, or the absolute number of eosinophils is greater than 100/µl effluent, eosinophilic peritonitis (EP) is diagnosed (93). This type of illness is less common and associated with a

more benign outcome than those resulting from bacterial contamination, and often resolves without any therapeutic treatment, and therefore makes it important to distinguish (94). The reasons for eosinophilic peritonitis are uncertain. Allergic reactions to some of the dialysis components, higher serum Immunoglobulin E (IgE) levels and Vancomycin induced EP have been described in the literature (93, 95).

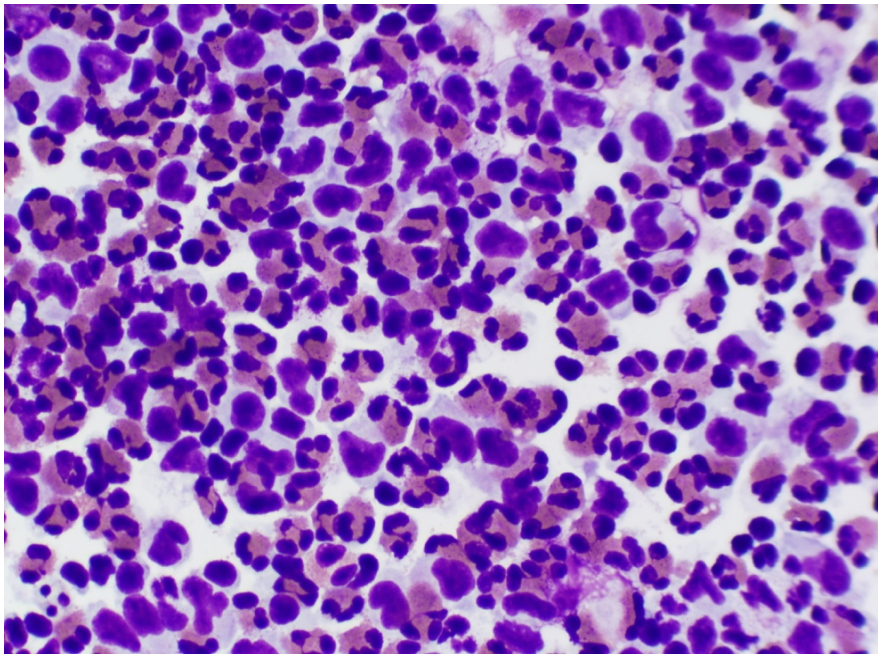

Figure 5: Peritoneal dialysate effluent with eosinophil granulocytes and lymphocytes (Source: Juliana Buchgraber, Medical University of Graz Division of Nephrology, Department of Internal Medicine)

Figure 5 is a capture of an eosinophilic culture-negative peritonitis episode.

Furthermore, diseases like appendicitis and perirectal abscess and fulminant sclerosing peritonitis were described to initially present like culture-negative peritonitis and rare antecedents, such as splenic infarction, were reported in the literature (96-99).
Regarding pathogens, gram-negative bacteria seem to play a minor part. In a study, conducted in 2008, Hausmann et al. compared Limulus amebocyte lysate in two groups, one with culture-negative episodes and one with gram-negative episodes as control group. Limulus amebocyte lysate (LAL) is extracted out of blood cells of the Atlantic horseshoe crab and reacts with the endotoxin of gram-negative bacteria, which is found in the cell wall and known as Lipopolysaccharide and is therefore usable for the detection of gram-negative bacteria (100). They came to the result that in 117 episodes of gram-negative peritonitis, 68% (n=80) were LAL positive and none of the 21 culture-negative peritonitis showed LAL positivity, which suggests that gram-negative bacteria is hardly involved and

initial antibiotic coverage of gram negative might be dropped (101). In order to achieve the best possible therapy for each patient, it is fundamental to isolate the causing pathogen.

In our study we tried to find out if our center matches the criteria of the ISPD guidelines regarding the PD-associated peritonitis rate, respectively the rate of culture negative peritonitis and whether effluent cytology, performed as Gram's stain, Hemacolor stain and microscopic survey of the dialysate, has an impact on diagnosis of peritonitis, and should therefore be considered in the diagnostic procedure.

2 Methods

2.1 Study population

This retrospective, monocentric study included all patients, who underwent at least one type of peritoneal dialysis between 01.01.2007 and 31.12.2017 at the outpatient department for peritoneal dialysis, Department of Nephrology, Medical University of Graz, Austria. We used the OEDTR (Österreichisches Dialyse- und Transplantationsregister), a register platform, including voluntarily Austrian patients undergoing renal replacement therapy and compared it with data from the local medical data system, MEDOCS (Steiermärkisches medizinisch-pflegerisches Dokumentations- und Kommunikationsnetzwerk), to select the patient collective. The OEDTR register contained 361 peritoneal dialysis patients of which 108 were excluded, because these patients were treated at the PD unit beyond our considered observational period. Therefore, we included 253 patients to retrospectively reevaluate their PD therapy. Because of the retrospective analysis of data, there are no age limits. Subsuming, the collective includes 250 patients with chronic kidney disease (CKD) stage 5 undergoing at least one method of PD and three individuals who were treated with ascites drainage, therefore using the same catheter type and being managed similarly to PD patients at the outpatient department for peritoneal dialysis.

To ascertain if patients suffered from peritonitis, we looked up their laboratory markers, in which, the mechanical and the hematological-cytological measured leucocytes are listed chronologically. If the number of leucocytes in the effluent exceeded 100/µL and at least one of the two further conditions for peritonitis was found in medical reports, matching the date on which elevated leucocytes were found, the peritonitis episode was added to survey. The medical reports showed if patients were diagnosed with peritonitis, got inpatient treatment or were treated outpatient. We then extracted information from the local medical

data system, MEDOCS, of laboratory results, doctors' letters and other documents with the support of the Institute for Medical Informatics, Statistics and Documentation of the Medical University of Graz. We collected data, containing demographic content such as age, gender, but also peritoneal dialysis specific parameters like age at PD start, underlying renal disease, time of catheter implantation, and peritonitis characteristics such as symptoms at diagnosis, time of inpatient treatment, initial cell count of leukocytes and erythrocytes, causing pathogen and switch to hemodialysis. Furthermore, we looked up the cytological report of the Department of Nephrology, which provides information about Gram's stain results in detail as well as culture results of the dialysate effluent cultivated in the microbiological laboratory of the Medical University of Graz.

2.2 Statistical analysis and literature research

For statistical analysis, we used IBM SPSS Statistics 25 via the Citrix Server of the Medical University of Graz and Microsoft Excel. The collection of all relevant information was carried out in Microsoft Excel. We performed a descriptive statistical analysis of the data we acquired and presented the information as median, mean value, total value and percentage. Plausibility was reviewed for each patients' data.

Via SPSS we also conducted a Kaplan Meier analysis to present survival function, respectively peritonitis free survival, for both genders as well as for diabetes mellitus versus non-diabetes mellitus patients.

Furthermore, we used the Kolmogorov-Smirnov-Test for testing the normal distribution and Mann-Whitney-U test, respectively t-test, for analyzing differences, for significance.

For the literature research, we used PubMed, a library for biomedical literature. We conducted our search using MESH terms such as "Peritonitis" AND "Peritoneal Dialysis" AND "Risk Factors", combined with keywords such as "eosinophilic" or "culture-negative peritonitis".

The patient cohort was split into the different types of therapies, which were managed at the PD unit. We split the cohort into patients, who only have a catheter due to ascites drainage and are therefore called "ascites only" and usual PD patients, hence called "PD-only". We further split the patient cohort "PD only" in two groups, videlicet a group of patients who did never suffer from peritonitis and one with patients who did sustain at least an episode of peritonitis.

2.3 Ethical aspects and benefits

On 19th of November 2017, the ethical committee of the Medical University of Graz confirmed the arrival of the application "Influence of higher intraperitoneal pressure to peritoneal dialysis associated rate of peritonitis – a retrospective study" and approved it on 22nd of January 2018. This study is part of the above mentioned, whereby the ethical committee did not raise an objection against the diploma thesis (EK-Number 30-097 ex 17/18). In order to fulfill the rules of medical data protection, patients' data was anonymized and password-protected. The benefit of this trial is expected for future patients treated with a type of peritoneal dialysis, in terms of finding the correct diagnosis and choosing the right therapy for patients who suffer from peritonitis, not only in Graz, but also in other PD units across the world.

2.4 Evaluation of peritoneal dialysis effluent diagnostic

2.4.1 Evaluation of peritoneal dialysis effluent cytology

In the outpatient Department for PD in the Medical University of Graz, patients present every four to six weeks at the department, except there are complaints occurring in the meantime, which force the patients for earlier consultation. Within these routine controls, every patient's peritoneal dialysis effluent is evaluated via cytology. During their visit, dialysate gets routinely drawn in an empty bag after at least 2 hours of dwell time of which then a sample is drained in a lithium-heparin-coated container (Vacuette® LH Lithium Heparin 6 ml, green/black). Also, according to the SOP (Standard Operating Procedure), if patients evolve symptoms of peritonitis, they are requested to bring the last (cloudy) bag with them to the department for evaluation.

For microscopic examination, whether Hemacolour or Gram staining is performed, four-hundred µl of the effluent are pipetted in a single cytotunnel™ where the tube is connected to an object slide in a Thermo Scientific™ Cytospin™ 4 Cytocentrifuge, as shown in Figure 6. If the dialysate effluent seems to be abundant in number of cells, respectively cloudy, only 200µl or 100µl are used. The sample is then set to 1000 rounds per minute for 10 minutes.

Figure 6: Thermo Scientific™ Cytospin™ 4 Cytocentrifuge

At the same time, mechanical/automated analysis of the dialysate effluent is performed using the Sysmex UF-1000i (Sysmex Austria®). Therefore, one thousand µl of the effluent, previously drawn into the lithium-heparin-coated container, are used.

For Hemacolour Staining, 4 steps are conducted:
First, the microscope slide is immersed for one minute in a methanol-containing solution (Hemacolor® Rapid Staining of Blood Smear, Solution 1: fixing solution, by Merck, 1.11955), directly followed by a one minute bath with a red colour reagent (Hemacolor® Rapid Staining of Blood Smear, Solution 2: Colour reagent red, by Merck, 1.11956). Following this, the slide undergoes a one-minute bath with a blue colour reagent (Hemacolor® Rapid Staining of Blood Smear, Solution 3: colour reagent blue, by Merck, 1.11957). Finally, the slide is steeped in a buffer solution for one minute, whereby the buffer solution is established with 1 buffer tablet (Microscopy Buffer Tablets pH 7.2, by Merck) dissolved in 1 liter of bi-distilled water. The microscopic examination of the hemacolour stained effluent is done with Olympus BH-2 microscope. The low powerfield (x 100 magnification) gives an overview of distribution, while the high powerfield (x 500

magnification) allows discrimination of cell types, mainly neutrophil granulocytes, eosinophil granulocytes, mesothelial cells, reactive mesothelial cells and erythrocytes.
If the cell count exceeds 50 leucocytes, additional Gram stain is performed. To perform Gram's staining, eight steps are performed, using Solutions by Merck®, Germany. First, moistening the microscope slide with Gram's crystal violet solution for one minute. After tipping the microscope slide to let the solution gutter from the microscope slide, Lugol's solution is moistened on the slide for one minute, followed by a bi-distilled water wash and a prolonged wash with Gram's decolorizing solution. When all blue residues are washed away and another wash with bi-distilled water is executed, Safranine solution is stained for one minute. After another wash with bi-distilled water and air drying the slide, microscopic examination of the Gram stained effluent is performed with an Olympus BH-2 microscope. While the low powerfield (x 100 magnification) gives an overview of distribution, the high powerfield (x 500 magnification) allows the intensive search for bacteria or other infectious organisms. For a complete microscopic examination of a Gram stained microscopic slide, all fields of vision must be screened. A mean number of fields of vision contains about 230 fields.

.

Figure 7: Staining substances: white=methanol, orange=color reagent red, violet= color reagent blue, light blue=buffer

This procedure is only made on weekdays and only during PD unit business hours. In cases, when effluent diagnostic is needed after business hours and on weekends, effluent mechanical analysis of the dialysate effluent is performed via the routine laboratory with a limited type of cytology.

2.4.2 Evaluation of peritoneal dialysis effluent culture

When patients visit the PD unit, nurses not only draw dialysate after 2 hours of dwell time for cytological examination into an empty bag, but also into a rapid blood culture bottle

bedside for culture cultivation, which is then placed on a storage container, to be picked up by the messenger service which is sent directly to the microbiological laboratory in the hospital.

2.4.3 Empirical therapy and antibiotic prescription

Patients are obliged to present immediately at the unit when complaints about symptoms emerge and are then advised to finish the dialysate exchange at home und bring the last (cloudy) effluent bag with them along. At the department, not only the brought along bags get examined, but also a fresh bag after 2 hours of dwell time is added to survey as well as the exit site is also object of investigation.

Empirical antibiosis at the unit for peritoneal dialysis, is routinely performed with Vancomycin and Ceftazidime (SOP). The initial loading dose consists of Vancomycin 20mg/kg bodyweight i.p., plus Ceftazidime 500 mg i.p., plus 1000 International Units (IE) heparin per liter i.p. In order to react quickly to possible reactions to Vancomycin, the running-in is fragmented to an overall dwell time of 6 to 8 hours. Alternatively, Cefazolin 500mg i.p., is installed instead of Vancomycin. If Vancomycin is the choice of treatment, 0.5 to 1.0 gram of the antibiotic is installed all 3 days with a through level of 15-20mg/l during the night bag. Further treatment consists of 125 mg Ceftazidime per liter dialysate and one thousand IE of heparin per liter of dialysate, which are administered under standard condition of CAPD regime with 4 exchanges. Patients treated with APD, are generally switched to CAPD during peritonitis episode. If there is no improvement after 5-7 days, antibiotic regimen should be reevaluated and adapted to culture result and, if necessary, catheter explantation is considered.

3 Results

After matching our inclusion criteria, a total of 253 patients were included in our study, of which 250 performed a type of peritoneal dialysis and 3 patients were only treated with ascites drainage. In Table 1, patient characteristics of both groups are shown.

		PD-only		Ascites-only	
Study population		250	*98.8%*	3	*1.2%*
Gender	male	169	*67.6%*	2	*67%*
	female	81	*32.4%*	1	*33%*
Average age at PD start (years)		56.5		46.1	
PD Duration	**Average PD duration per patient (months)**	31.82		23.41	
	Average PD duration per patient (days)	967.57		711.67	
Underlying renal disease					
	Diabetic nephropathy both types	43	*17.2%*	0	*0%*
	Diabetic nephropathy in type I diabetes	12	*4.8%*	0	*0%*
	Diabetic nephropathy in type II diabetes	31	*12.4%*	0	*0%*
	Chronic hypertensive nephropathy	35	*14.0%*	0	*0%*
	Autosomal dominant (AD) polycystic kidney disease	19	*7.6%*	0	*33%*
	IgA nephropathy	15	*6.0%*	0	*0%*
	Atheroembolic renal disease/ ischemic nephropathy	13	*5.2%*	0	*0%*
	Primary focal segmental glomerulosclerosis	7	*2.8%*	0	*0%*
	Cardiorenal syndrome	6	*2.4%*	2	*67%*
	Systemic lupus erythematosus / nephritis	5	*2.0%*	0	*0%*
	Nephropathy due to analgesic drugs	5	*2.0%*	0	*0%*
	Primary reflux nephropathy - sporadic	5	*2.0%*	0	*0%*
	Idiopathic rapidly progressive glomerulonephritis	4	*1.6%*	0	*0%*
	Ischaemic nephropathy / microvascular disease	4	*1.6%*	0	*0%*
	Thrombotic thrombocytopenic purpura	1	*0.4%*	0	*0%*
	Others	88	*35.2%*	0	*0%*

Table 1: Patient characteristics 1

Further information is given in the following chapter 3.1, which concentrates on the 250 patients undergoing peritoneal dialysis. For results of the 3 patients, who were only treated with ascites drainage, see chapter 3.2.

3.1 Patients undergoing peritoneal dialysis

3.1.1 Patient characteristics in patients undergoing peritoneal dialysis

The percentage of women among the patient`s population was 32.4% (81/250). Regarding gender, women tended to be slightly younger and stay marginally longer on PD therapy. The mean time under therapy was 967.6 ± 695.1 days under therapy, with 936.6 days for men (95% CI: 888.40 – 1039.79; range: 12 – 3809) and 1032.3 days for women (95% CI: 871.65 – 1192.84; range: 15 – 3412).

The mean age at PD-Start for men was 57.37 ± 14.12 years (21.82 – 86.59) and 53.54 ± 16.91 years (18.81 – 86.13) for women. As shown in Table 1, the most common underlying renal disease was found to be diabetes mellitus with 17.2%, of that 12.4% with diabetic nephropathy in type 2 diabetes and 4.8% with diabetic nephropathy in type 1 diabetes, respectively, followed by chronic hypertensive nephropathy (14%), autosomal dominant polycystic kidney disease (7.6%), IgA nephropathy (6%), artheroembolic renal disease/ischemic renal disease (5.2%), primary focal segmental glomerulosclerosis (2.8%), cardiorenal syndrome (2.4%) systemic lupus erythematosus nephritis (2%), nephropathy due to analgesic drugs (2%), primary reflux nephropathy (2%), idiopathic rapidly progressive glomerulonephritis (1.6%), ischemic nephropathy/ microvascular disease (1.6%) and thrombotic thrombocytopenic purpura. The remaining 88 underlying renal diseases (35.2%) consisted of other underlying diseases.

3.1.2 Peritoneal dialysis associated Peritonitis

In a total of 250 patients undergoing peritoneal dialysis, 155 episodes of peritonitis were detected in 95 patients during the observational period. In the remaining 155 patients, no infection could be detected. As shown in Figure 8, in 60 individuals only one episode of peritonitis occurred. In 22 patients a total of 2 episodes of peritonitis occurred, while in 9 patients 3 episodes, in 1 patient 4 episodes, in 2 patients 5 episodes and in 1 person 10 episodes happened to be. The calculation and illustration of the episodes were made, based on the guidelines mentioned in chapter 1.1.3.1.1 and 1.1.3.1.3.

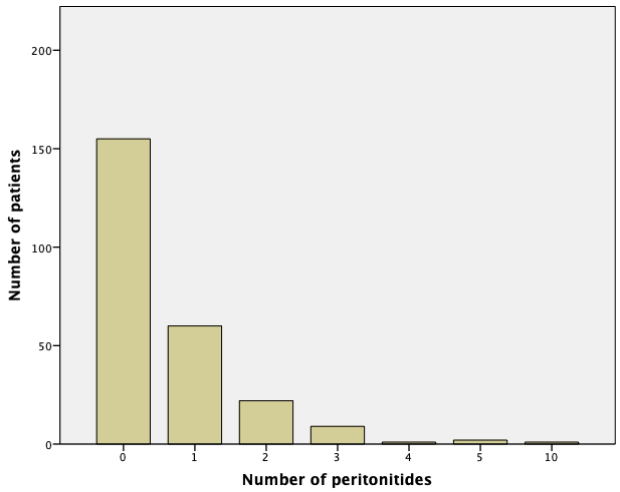

Figure 8: Distribution of number of peritonitis

3.1.3 Peritoneal dialysis associated Peritonitis rate

We considered episodes which matched 2 of the 3 criteria according to the ISPD Guidelines (8) see 1.1.3.1.1., diagnosis of peritoneal dialysis associated peritonitis. Elaborating all PD days of all PD patients under risk, in other words undergoing PD, the peritonitis rate was calculated in different descriptions as follows.

- **Centre-referential calculation of peritonitis rate as episodes per patient year (target value < 0.5/year):** The centre-referential rate values 0,2339 (155/662,72055 years) episodes per patient year.

- **Centre-referential calculation of peritonitis rate as percentage of patients per period of time who are peritonitis free**: 62.0% of the patients were never compromised by an episode of peritonitis.

- **Patient specific calculation of peritonitis rate as 1 episode per number of patient months (target value: < 1 episode/24 months):** 1 episode every 51.19 patient months.

- **Patient specific calculation as episodes per year (target value: < 0.5/year):** 0,30326 episodes per patient year.

3.1.4 Patient characteristic in Peritonitis Patients

Patient characteristics differed, but statistically insignificant, from their peritonitis behavior according PD duration, as shown in Table 2. The mean age of patients at the start of PD who at least once suffered from peritonitis was 56.6 ± 15.02 and 55.8 ± 15.28 years (p= 0.693), for patients who did not. While peritonitis free patients had undergone a mean PD duration of 26.3 ± 18.5 months, patients with peritonitis had undergone a mean PD duration of 40.9 ± 26.3 months (p=0.000).

Women slightly tend to have more often at least one episode of peritonitis (41.9%; 34/81 vs. 36.0%; 61/169; p=0.370). Women also seem to have earlier episodes of peritonitis, reflected in a 12-month and 24-month eventless time (defined as at least 1 episode of peritonitis) of 88,8% for men versus 83,6% for women, respectively 76,9% for men versus 68,0% in women, but according to the Mann-Whitney-U-test, the difference in time to first peritonitis in months is not significant (p= 0.825) . After 3 years tendencies disappear.

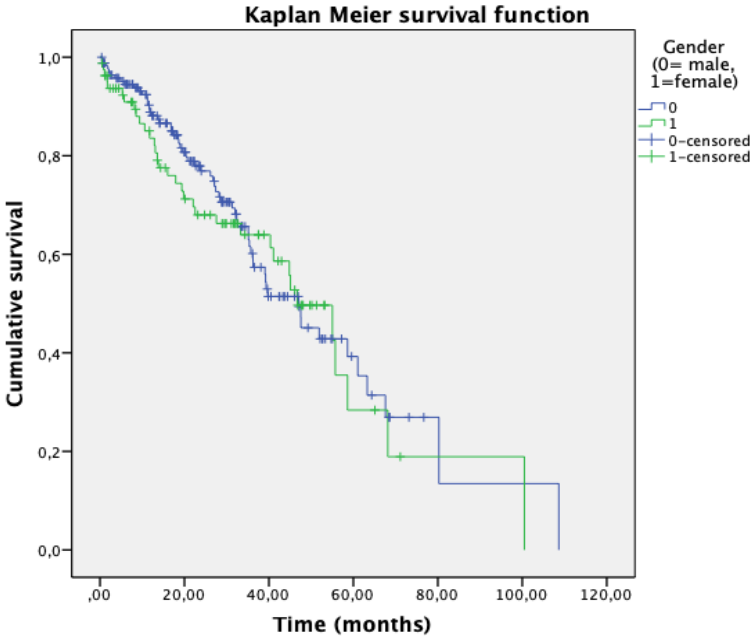

Figure 9: Survival function regarding gender

The percentage of PD patients with diabetic nephropathy, who had at least one episode of peritonitis (37.2%, 16/43), did not exceed the non-diabetic study population (38.2%, 79/207) (p= 0.907). Patients with diabetes mellitus suffered earlier from episodes of peritonitis compared to non-diabetic patients. After 12 months, 88.7% of non-diabetic patients and 77.6% of diabetic patients were free of peritonitis at this time, but after 24 months, the proportion approximated with 73.9% of peritonitis-free non-diabetic patients and 74.8% peritonitis free diabetics, see Figure 10.

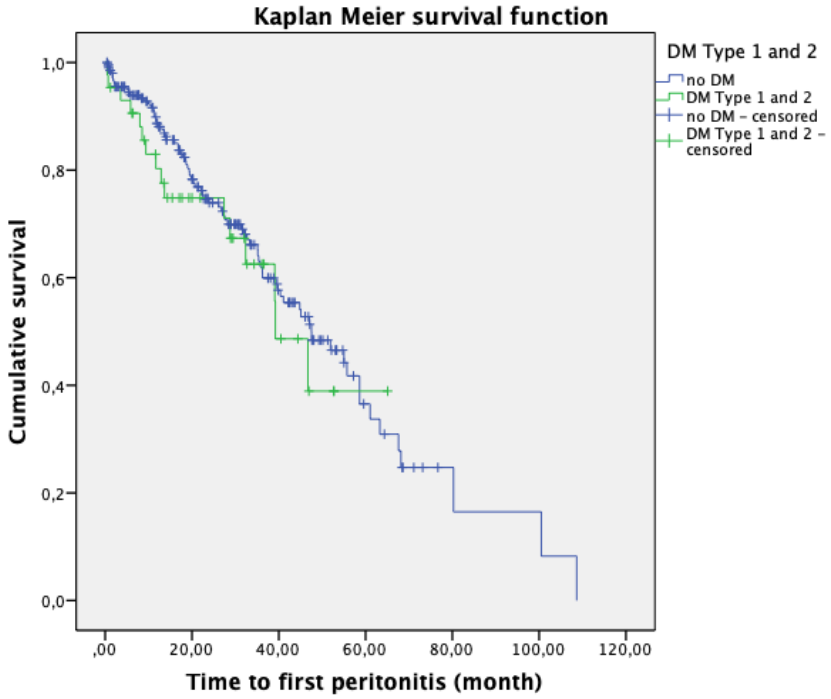

Figure 10: Survival function regarding diabetes mellitus

The percentage of peritonitis in patients with autosomal dominant polycystic kidney disease was increased (47%, 9/19), compared to the non-ADPKD study population (37.2%, 86/231) (p= 0.766).

Also, patients with thrombotic microangiopathy and IgA nephropathy showed higher prevalence in peritonitis, compared to the non-thrombotic microangiopathy and non-IgA nephropathy population (46.2%, 6/13 vs. 37.6%, 89/237; 40.0%, 6/15 vs. 37.8%, 89/235).

In the remaining patients with rare underlying renal diseases, trends were not considered reliable, because of the small number of patients in the individual entity.

		Patients with Peritonitis (n=95) (38%)	Patients without Peritonitis (n=155) (62%)	P-values (α=0.05)
Gender				*0.371*
	male	61 *(64.2%)*	108 *(69.7%)*	
	female	34 *(35.8%)*	47 *(30.3%)*	
Average age at PD start (years)		56.6 (SD 15.02)	55.8 (SD 15.28)	*0.742*
PD Duration	**Average PD duration per patient (months)**	40,92 (SD 26.27)	26,25 (SD 18.49)	*0.000*
	Average PD duration per patient (days)	1244,01	798,14	
Underlying renal disease				*0.239*
	Diabetic nephropathy both types	16 *(17.8%)*	27 *(17.4%)*	
	Diabetic nephropathy in type I diabetes	3 *(3.2%)*	9 *(5.8%)*	*1.000*
	Diabetic nephropathy in type II diabetes	13 *(13.7%)*	18 *(11.6%)*	*1.000*
	Autosomal dominant (AD) polycystic kidney disease	9 *(9.5%)*	10 *(6.5%)*	*1.000*
	Chronic hypertensive nephropathy	12 *(12.6%)*	23 *(14.8%)*	*1.000*
	Atheroembolic renal disease/ ischemic nephropathy	6 *(6.3%)*	7 *(4.5%)*	*1.000*
	IgA nephropathy	6 *(6.3%)*	9 *(5.8%)*	
	Idiopatic rapidly progressive glomerulonephritis	2 *(2.1%)*	2 *(1.3%)*	
	Primary focal segmental glomerulosclerosis	2 *(2.1%)*	5 *(3.2%)*	
	Systemic lupus erythematosus / nephritis	1 *(1.1%)*	4 *(2.6%)*	
	Nephropathy due to analgesic drugs	2 *(2.1%)*	3 *(1.9%)*	
	Ischaemic nephropathy / microvascular disease	2 *(2.1%)*	2 *(1.3%)*	
	Primary reflux nephropathy - sporadic	0 *(0%)*	5 *(3.2%)*	
	Cardiorenal syndrome	0 *(0%)*	6 *(3.9%)*	
	Others	37 *(38.9%)*	52 *(33.5%)*	

Table 2: Patient characteristics 2 - according to peritonitis behavior

3.1.5 Symptoms

Forty-one patients presented with two or more symptoms, as shown in Table 3, resulting in a total of 201 described symptoms, and therefore as a consequence the percentage of episodes sums up to more than 100%. Besides the most common symptom pain (42.3%, 85/201), cloudy effluent mount up to about one quarter of reported symptoms (27.9%, 56/201). Clear effluent with only fibrin strings in the effluent summed up to 1.0% (2/201) while one patient mentioned troubles with the drainage of dialysate effluent (0.5%, 1/201). Furthermore, vomiting and diarrhea were reported in 5.5% (11/201) and 4.0% (9/201), respectively. Patients mentioned fever in 4.5% (9/201) of reports, before applying in the outpatient department.

Symptoms	Number (n=201)	Percentage of reports	Percentage of episodes
Pain	85	*42,3%*	55%
Cloudy effluent	56	*27,9%*	36%
Vomiting	11	*5,5%*	7%
Diarrhea	8	*4,0%*	5%
Fever	9	*4,5%*	6%
Troubles with the effluent	1	*0,5%*	1%
Fibrin strings	2	*1,0%*	1%
None	7	*3,5%*	5%
No data	22	*10,9%*	14%

Table 3: Symptoms of all PD patients

Patients' symptoms occurred on an average of 1.34 days (range: 0 – 14; 95% CI: 0.82039 – 1.86926) before showing up at the outpatient unit for peritoneal dialysis and being diagnosed with peritonitis. Only in 87 episodes, the time between the first occurrence of symptoms to the administration to the PD-unit could be assessed. Interestingly, there was a difference in the undergoing type of PD regarding symptom occurrence, which was statistically not significant. Patients, undergoing APD, presented 1.62 days after first occurrence of symptoms while patients treated with CAPD visited the outpatient department 0.871 days after first presentation of complaints (63.2%, 55/87 in APD/CCPD and 35.6%%, 31/87 in CAPD; p=0.208). One episode, in which the occurrence of symptoms could be assessed, occurred during treatment during treatment with hemodialysis (1,1%, 1/87).

3.1.6 Temporal Occurrence

The median time from PD-start to the first episode peritonitis was 803.92 days ± 661.241 (range: 14 – 3303; 95% CI: 669.21– 938.62), respectively approximately 2 years and 2 months, but with a wide distribution, see Figure 11.

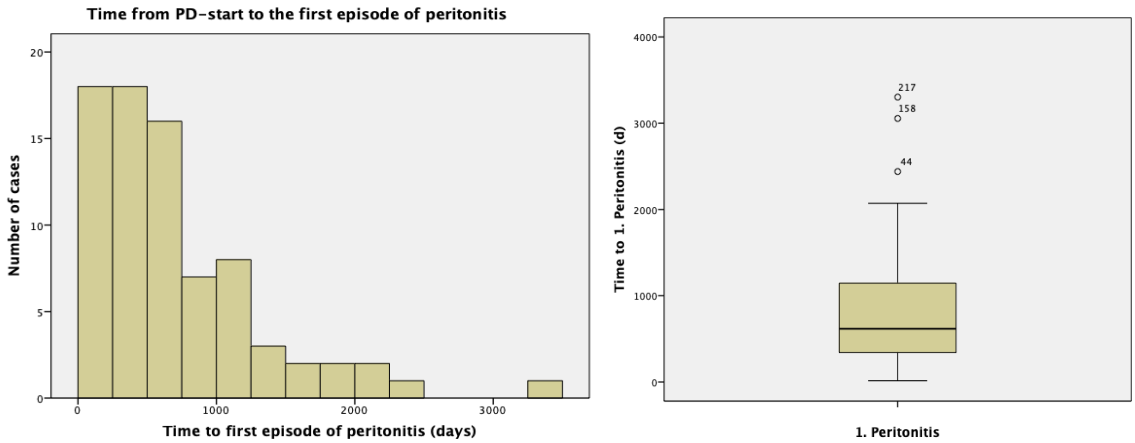

Figure 11: Time to first peritonitis (days)

3.1.7 Temporal distribution

Absolute episodes of PD associated peritonitis undulated from a minimum of 4 to a maximum of 23 with median of 14.09 ± 3.723 episodes per year. In Figure 12, absolute peritonitis rates are shown between the years 2007 and 2017. Of course, this data is not standardized to the number of patients under risk or number of patient years under risk.

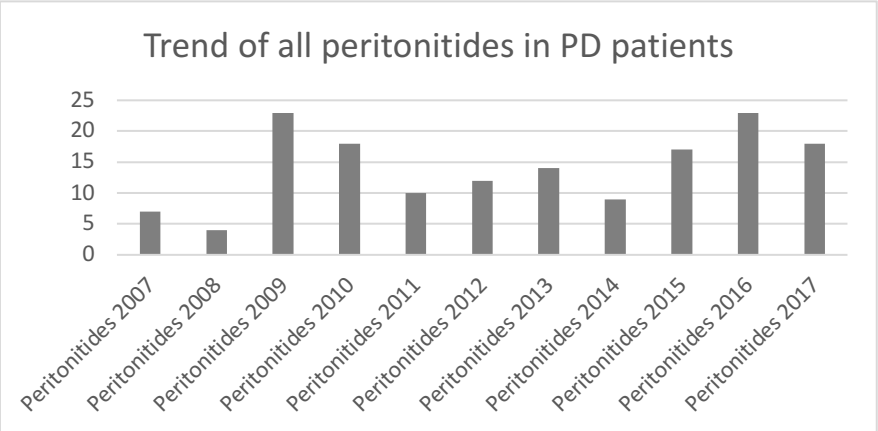

Figure 12: Absolute peritonitis episodes (2007-2017)

Taking the number of patients and their years under risk into account, peritonitis rate varied from 0.08433 to 0.38401 (0.23176 ± 0.0920) episodes per year. In 2009 and 2010 as well as in the years 2016 and 2017 the rate seems to peak, but still are below the ISPD recommendation of 0,5 episodes per year.

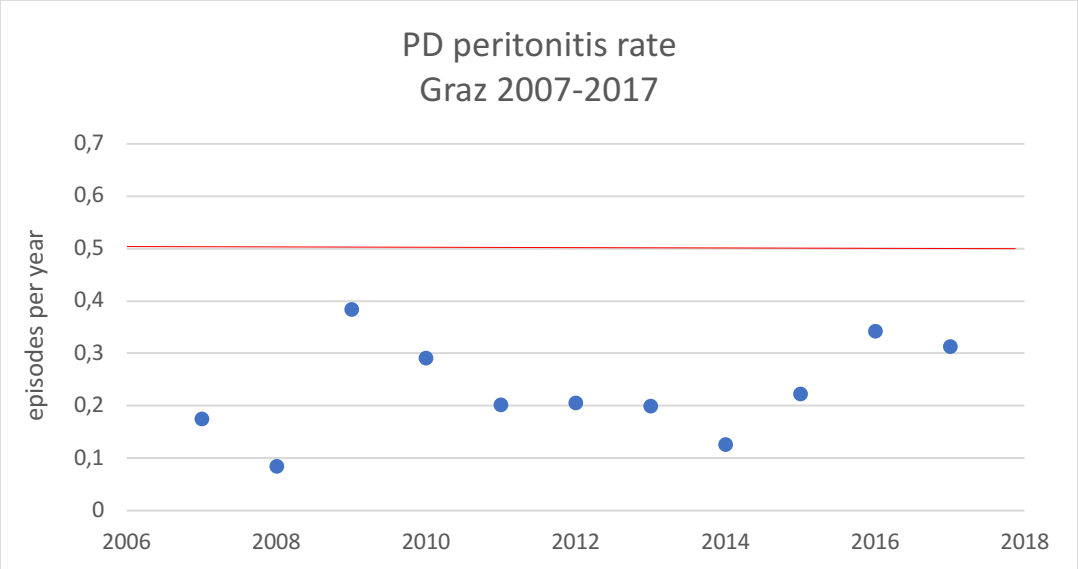

Figure 13: PD associated peritonitis rate (2007-2017)

The distribution of peritonitis regarding months of the year was throughout January to May comparable. Interestingly, we observed a peak in July, and the lowest incidence in August and September.

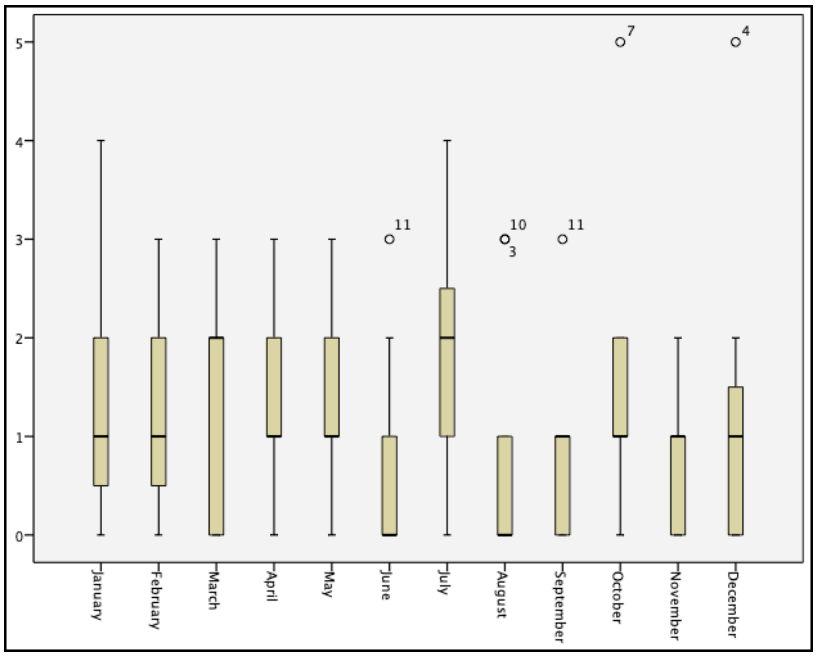

Figure 14: Distribution of peritonitis over months

As we focus on the years with higher prevalence of peritonitis rate, as in the years 2009 and 2010, the main peak was in the winter months, from December till March, while in 2016 and 2017 peaks were assessed in July, contributing and corresponding to the overall monthly trends, with low peritonitis rates in the winter months.

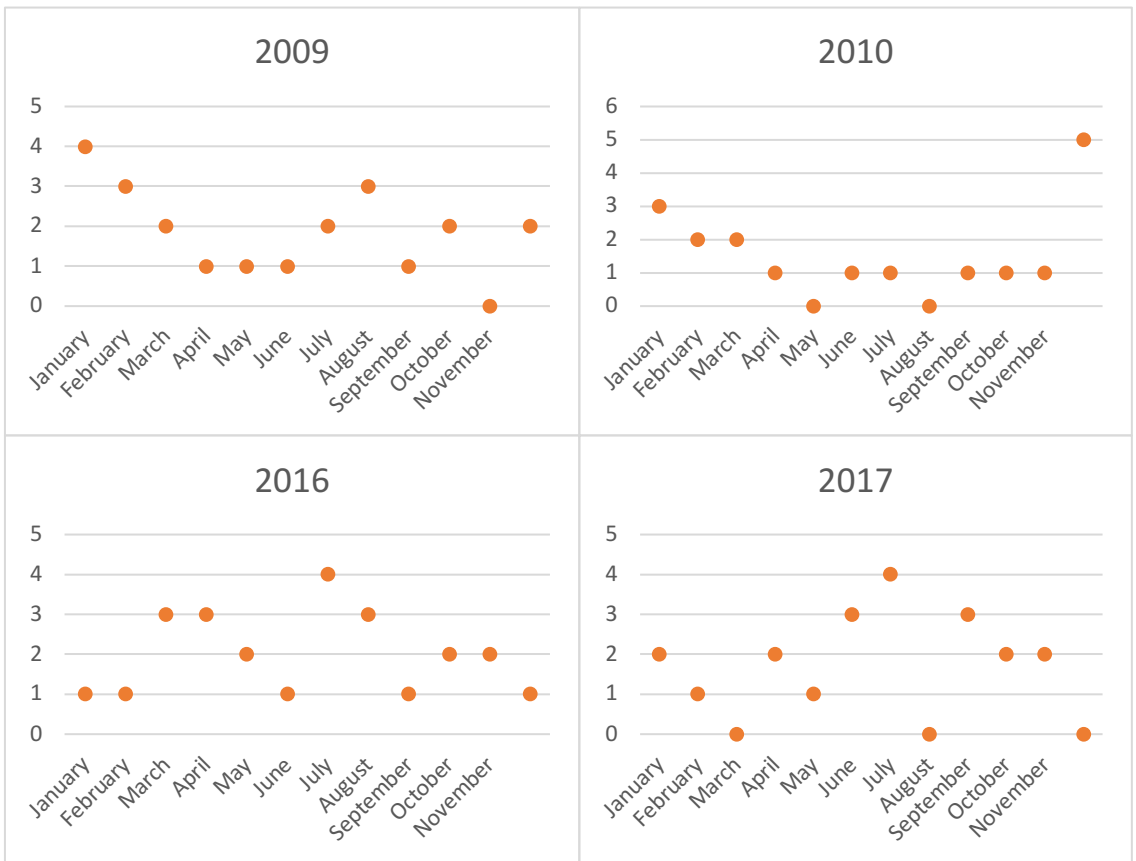

Figure 15: Distribution of peritonitis over months regarding years

Out of 41 CNP, 26.8% (11/41) of CNP were found to occur on Mondays. Further on happened to be 12.2% (5/41) episodes on Tuesdays, 14.6% (6/41) on Wednesdays, 14.6% (6/41) on Thursdays, 21.9% (9/41) on Fridays, 2.4% (1/41) on Saturdays and 7.3% (3/41) on Sundays.

3.1.8 Peritoneal dialysis associated Peritonitis characteristics

In a total of 155 episodes, sixty-three (40.6%, 63/155) of the episodes were found to be under the treatment of CAPD, ninety (58.1%, 90/155) under APD, one patient (0.7%, 1/155) suffered peritonitis while performing temporary hemodialysis. In one patient (0.7%, 1/155) an episode of peritonitis happened while therapy was paused.

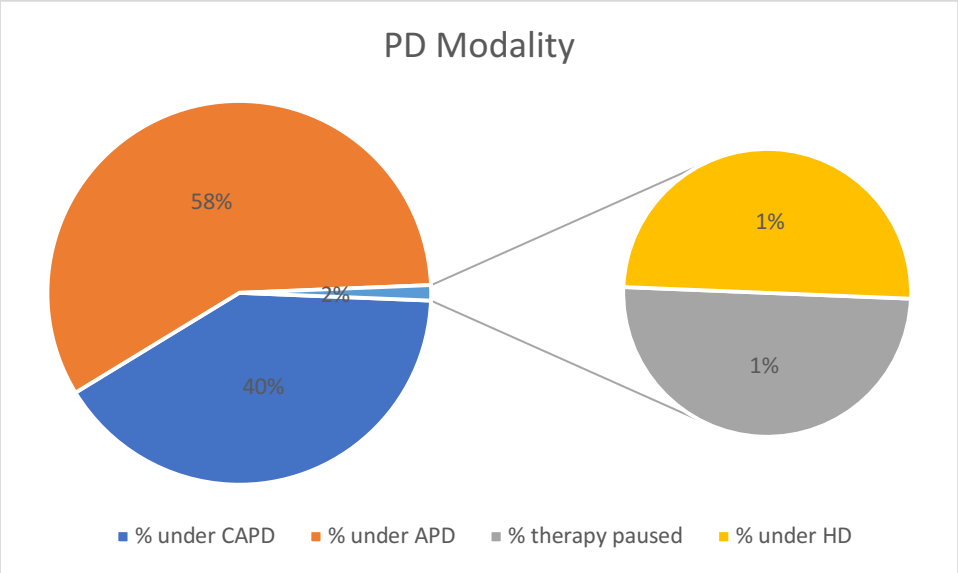

Figure 16: Peritonitis regarding PD modality

The relative numbers averaged 0.2518 episodes per year in APD/CCPD patients compared to 0.2238 per year in CAPD patients.

3.1.9 Outcome of peritonitis

3.1.9.1 Dialysate effluent leucocytes as response marker

The average of initial leukocytes in the dialysate effluent for the episodes of all PD patients (n=152) was 6950.88 leukocytes/µl (range: 28 – 94300; 95% CI: 5124.23319 – 8777.52996), while the average number of erythrocytes/µl was 334.14 (range: 0 – 8383; 95% CI: 165.4948 – 502.7959) resulting in a ratio of leukocytes to erythrocytes of 20.80. The reason why only 152 episodes instead of 155 were counted, was because in one case the effluent was doughy to such an extent, that the cell count could was not analyzable. In two cases, the initial cell count was not assessed in the MEDOCS System.

After initiating antibiosis, the mean time until leukocytes in the dialysate dropped below 100/µl were 7.19 ± 6.07 (95% CI: 6.13270 – 8.24525, range 0 – 30) days in the overall cohort.

When comparing the various entities, regarding causing organism, there was a different result. The culture-positive, gram-positive episodes showed the fastest response with 6.41 ± 5.337 days, while the group with various germs showed the worst response with 13.25 ± 9.032 days until leucocytes fell below 100/µl.

In the culture-positive group (n=93) the mean time was 7.12 ± 5.681 days (range 0 – 25; 95% CI: 5.95 – 8.29), as mentioned before, in the culture-positive, gram-positive cohort (n=69) 6.41 ± 5.337 days (range: 0 – 25; 95% CI: 5.12 – 7.69), in the culture-positive, gram-negative group (n=20) 8.35 ± 5.489 days (range: 1 – 19; 95% CI: 5.78 – 10.92), and in the culture-positive group with various germs (n=4) 13.25 ± 9.032 days (range: 4 – 21; 95% CI: (-1.12 – 27.62).

In the culture-negative group (n=34) time to effluent normalization was 7.29 ± 7.163 days (range: 1 – 30; 95% CI: 4.79 – 9.79). The reason the number of incorporated leucocytes normalization is smaller (n=127) than the number of peritonitis (n=155) is due to missing data.

3.1.9.2 Kidney replacement therapy changes

Nineteen patients (12.3%, 19/155) stopped peritoneal dialysis right after an episode of peritonitis. Five Patients (3.2%, 5/155) died during, respectively right after an episode of peritonitis due to cardiac arrest (n=4) or due to sepsis (n=1).

Fifty-two patients (20.8%) were still undergoing peritoneal dialysis at the end of the period of observation. Eighty patients (32.0%) had undergone a renal transplantation and 46 patients (18.4%) switched to hemodialysis (HD). The main reason to switch to hemodialysis was found to be peritonitis (36.9%, 17/46), but also due to PD-insufficiency (34.7%, 16/46) and leakage (10.8%, 5/46), see Figure 17 and 18.

Three patients (1.2%, 3/250) regained their kidney function without any need for further kidney replacement therapy (switched to conservative therapy). Forty-seven patients (18.8%) deceased during the observation period, under which only 5 patients (2%) died while having an episode of peritonitis, as mentioned above. In 22 patients (8.8%), the data was not traceable.

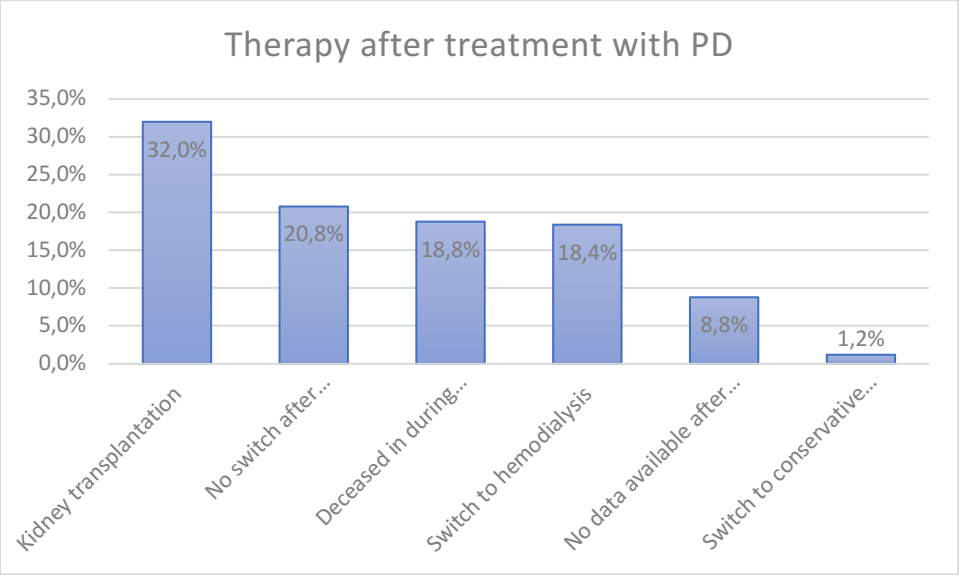

Figure 17: Kidney replacement therapy changes after the observational period with PD

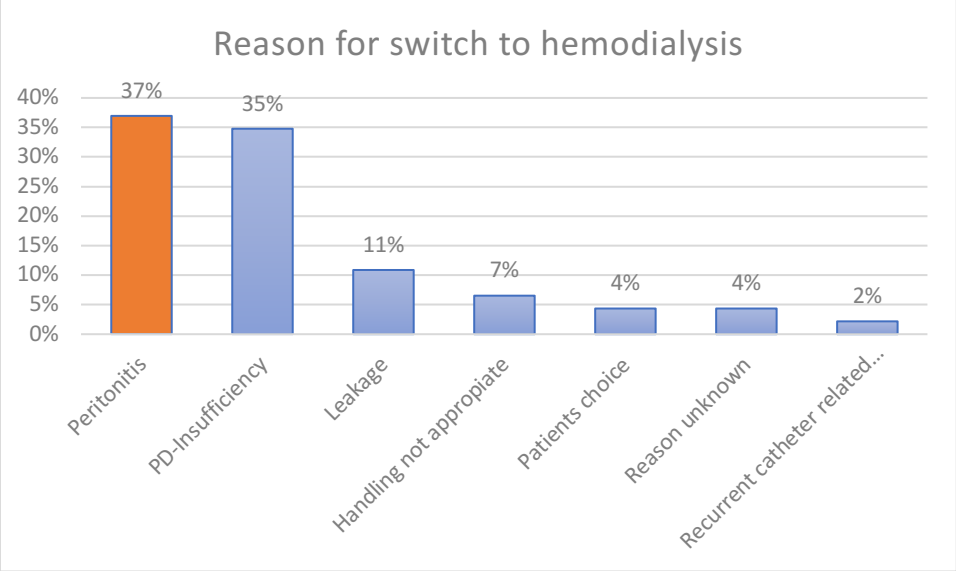

Figure 18: Reasons for switch to hemodialysis

3.1.9.3 Relapsing, recurrent and repeating episodes

We discovered 3 recurrent, 6 relapsing and 13 repeats of peritonitis in our study population, while relapsing episodes are not counted as another episode, see in 1.1.3.1.3 (11).

Pathogens causing relapsing episodes were Sphingomonas spp., E. coli, Achromobacter dentrificans, Hemophilus influenzae and CNS.

In the observational period, thirteen cases of repeat peritonitis occurred, while two patients had 4 times each a so-called repeat which account for 8 of 13 episodes. Nearly one half of

the repeat peritonitis episodes (46.2%, 6/13) were caused by Staphylococcus aureus, 3 (23.1%, 3/13) by Coagulase-negative Staphylococci, two were due to Enterococci and 1 each with Micrococcus sp. and Escherichia coli.

3.1.10 Peritoneal dialysis effluent diagnostic culture evaluation

Our collective included 250 patients, of which 95 patients suffered from at least one peritonitis and 155 did not. In total, 155 episodes occurred in 95 patients of which 114 could be linked to a causing organism. In 41 cases, the cultures remained negative throughout the treatment. The guidelines suggest a culture-negative rate below 15 percent (11).

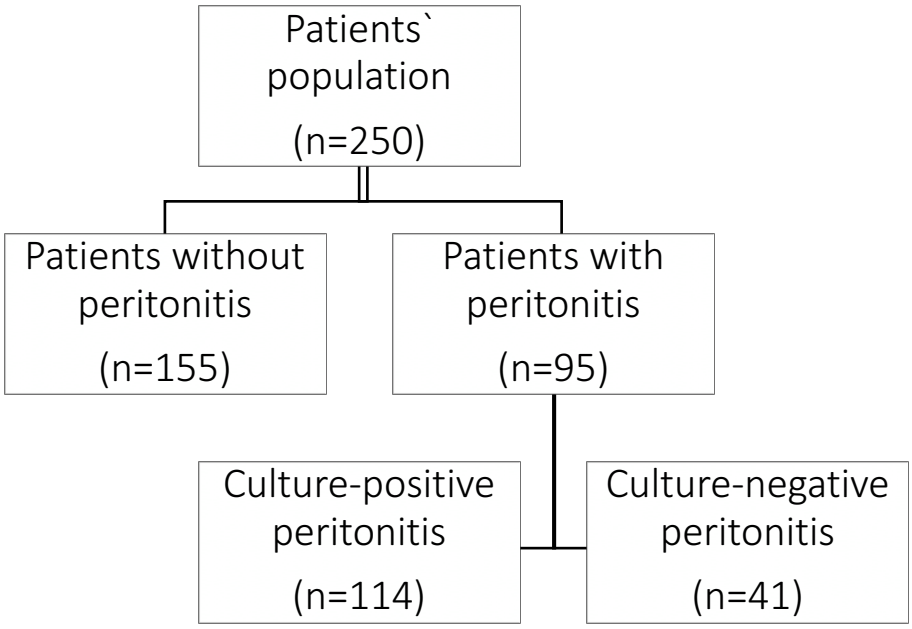

Figure 19: Flowchart of patient-tree

3.1.10.1 Dialysate effluent leucocytes regarding culture result

Compared to the culture-positive subgroup in which, the average initial leukocyte cell count was 8547.1/µl, the initial leucocytes in the group with culture-negative result were lower, at an average of 1538.9/µl.

3.1.10.2 Symptoms and outcome regarding culture result

Interestingly, the symptoms regarding culture behaviour varied. In episodes, in which a causing organism could be identified, the patients complained more often pain (45.7% vs. 32%), cloudy effluent (29.1% vs. 24.0%) and vomiting (6.6% vs. 2.0%), compared to the culture-negative group and also no symptoms were more likely to be found in episodes, in which a germ could be detected (4.0% vs. 2.0%). Missing data was more frequent in patients with culture-negative peritonitis (26.0% vs. 5.9%), see Table 4.

	Culture-positive peritonitis (n=114)		Culture-negative peritonitis (n=41)	
	Absolute number of reports(n=151)	Percentage of reports	Absolute number of reports (n=50)	Percentage of reports
Symptoms				
Pain	69	*45.7%*	16	*32.0%*
Cloudy effluent	44	*29.1%*	12	*24.0%*
Vomiting	10	*6.6%*	1	*2.0%*
Diarrhea	6	*4.0%*	2	*4.0%*
Fever	6	*4.0%*	3	*6.0%*
Troubles with the effluent	0	*0.0%*	1	*2.0%*
Fibrin strings	1	*0.7%*	1	*2.0%*
None	6	*4.0%*	1	*2.0%*
No data	9	*5.9%*	13	*26.0%*

Table 4: Symptoms regarding culture-behavior

The diagnosis peritonitis, in this one patient without symptoms and negative result of the culture can still be made, because the organism can also be verified via Gram's stain, which happened to be in this case.

When dividing the CNP group in the eosinophilic and neutrophilic and comparing with the CPP group (see Table 5), the results varied. When mainly eosinophils where found to be in the dialysate, not only initial leucocytes (568/µl vs. 1820/µl and 8346/µl), but also time until normalization was lower (6.5 days vs. 7.5 and 7.1 days) and switch to HD was less frequent (0 vs. 5 and 18 cases), compared to the other cohorts. In Table 5, the symptom pain is referred to the number of episodes (10 eosinophil CNP, 31 neutrophil CNP and 114 CPP) not the total number of reported symptoms which were mentioned in doctors' letters at presentation at the PD unit.

Culture	**negative**		**positive**
Cytology	eosinophil	neutrophil	neutrophil
Initial Leucocytes [/µl]	568	1820	8346
Normalization [days]	6.5	7.5	7.1
Symptom Pain [%]	40	38	61
Switch to HD [n]	0	5	18

Table 5: Symptoms regarding cytology-behavior

3.1.11 Culture-positive peritonitis

In our observation period, 114 culture-positive peritonitis occurred. In the years 2009 and 2016, we were able to observe a peak of absolute numbers, and in the years 2014 and 2012 the highest relative numbers (see Figure 20 and Table 6).

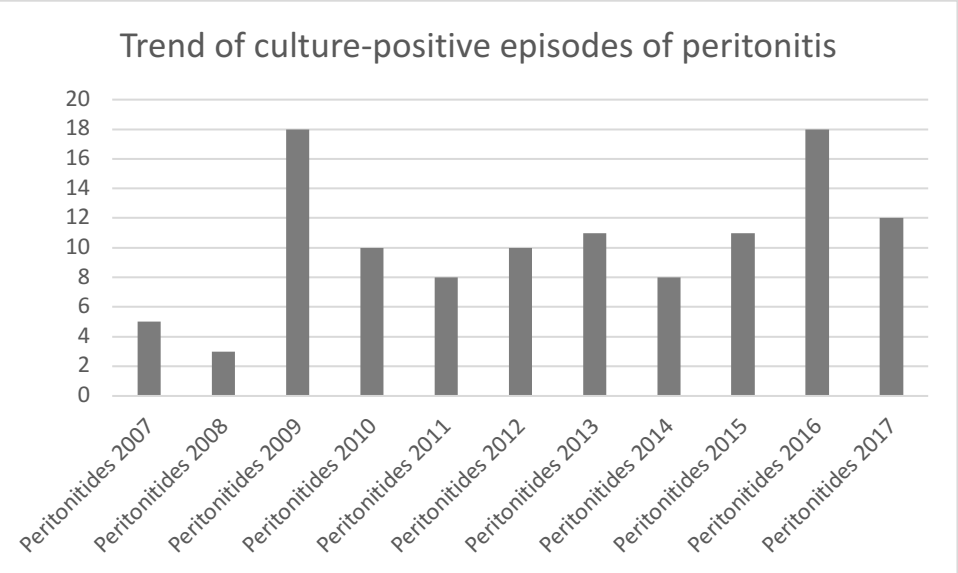

Figure 20: Absolute numbers and trend of culture-positive peritonitis

Year	Number of peritonitides (n=155)	Number of culture-positive peritonitides (n=114)	Percentage
2007	7	5	*71%*
2008	4	3	*75%*
2009	23	18	*78%*
2010	18	10	*56%*
2011	10	8	*80%*
2012	12	10	*83%*
2013	14	11	*79%*
2014	9	8	*89%*
2015	17	11	*65%*
2016	23	18	*78%*
2017	18	12	*67%*

Table 6: Relative numbers and trend of culture-positive peritonitis

3.1.11.1 Cultivated germs in Culture-positive (CPP) peritonitis

We identified 51 different germs throughout 114 culture-positive (CPP) episodes throughout the survey.

In every fifth CPP episode (21.9%, 25/114), two or more pathogens were cultivated, while in the remaining 89 episodes (78.1%, 89/114) only one germ was cultivated.

The following four most common pathogens contribute to 46.4% (64/138) of all germs found in CPP episodes, as there were S. aureus (21/138, 15.2%), Staphylococcus epidermis (18/138, 13.0%), E. coli (12/138, 8.7%) and CNS (13/138, 9.4%). Six episodes contribute to Enterococcus (6/138, 4.3%) and Streptococcus mitis (6/138, 4.3%). The remaining 45 pathogens compose of Streptococcus agalactiae (3/138, 2.1%), Staphylococcus hemolyticus (3/138, 2.1%), Micrococcus sp. (3/138, 2.1%), Candida albicans (3/138, 2.1%), Klebsiella oxytoca (2/138, 1.5%), Morganella morganii (2/138, 1.5%), Streptococcus sanguinis (2/138, 1.5%), Gemella morbillorum (2/138, 1.5%), Enterococcus faecium (2/138, 1.5%), Haemophilus influenzae (2/138, 1.5%), Streptococcus sp. (2/138, 1.5%), Enterobacter sp. (2/138, 1.5%). The remaining brown fraction "Others" in the left upper part in Figure 21 includes pathogens, that were always cultured only once, including Streptococcus pneumoniae, Providencia sp., Serratia marcescens, Staphylococcus caprae, Citrobacter freundii, Acinetobacter sp., Raoultella ornithinolytica, Dermabacter hominis, Corynebacterium sp., Aeromonas hydrophilia, Achromobacter dentrificans, Pasteurella sp., Proteus mirabilis, Streptococcus intermedius, Citrobacter braakii, Corynebacterium striatum, Abiotrophia adiacens, Bacteroides caccae, Fusobacterium mortiferum, Bacillus simplex, Bifidobacterium sp., Staphylococcus cohnii cohnii, Klebsiella pneumoniae, Staphylococcus lugdenensis, Lactoloccus lactis lactis, Acinetobacter Iwofii, Bacteroides vulgatus, Streptococcus equinus, Sphingomonas paucimobilis, Aurantimonas altamirensis, Staphylococcus hominis and Streptococcus acidominimus.

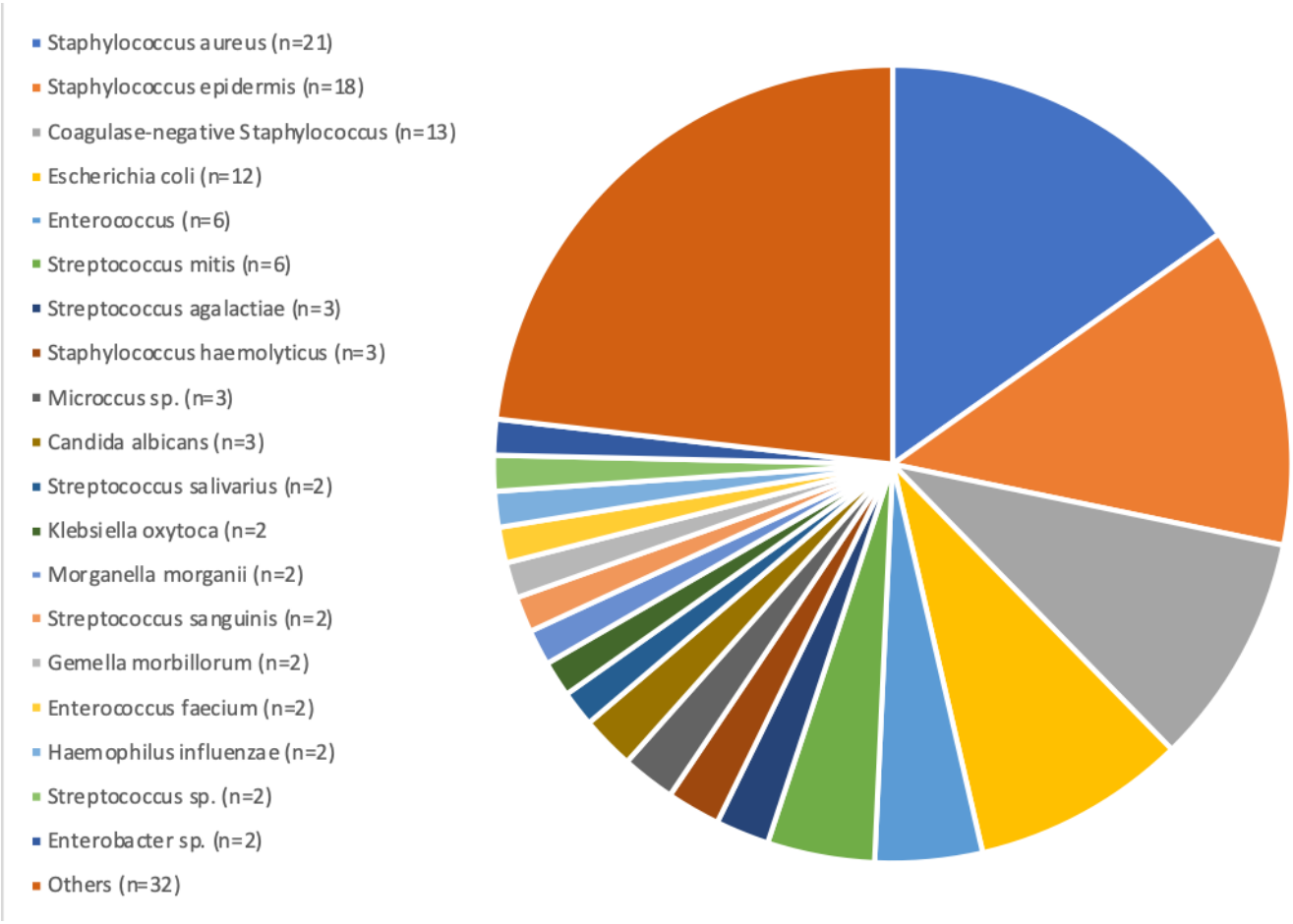

Figure 21: Causing organisms of peritonitis

3.1.11.2 Seasonal distribution of peritonitis and causing organisms

There was a different distribution of germs throughout the seasons of the year. Coagulase-negative Stahylococci (CNS) were in our collective found to be not involved in infections in autumn, while Corynbacterium was only observed during winter and other-grampositive cocci had a similar distribution throughout the year. Polymicrobial milieu was most often seen in spring, while culture-negative peritonitis (CNP) was most common in autumn.

Causing organism	Spring	Summer	Autumn	Winter
CNP	19%	18%	47%	23%
CNS	10%	8%	0%	13%
Gram-negative germs	10%	21%	8%	10%
Corynebacterium	0%	0%	0%	3%
Polymicrobial	29%	11%	8%	13%
Other gram-positive cocci	31%	37%	33%	33%
Other gram-positive germs	2%	5%	3%	3%
Fungi	0%	0%	0%	3%

Table 7: Seasonal distribution of peritonitis and causing organisms

3.1.11.3 Cytology in culture-positive peritonitis

As shown in Figure 22, in a total of 114 cases of CPP peritonitis, 3 cytology reports were not available (2.6%, 3/114) and in one case Gram's staining was not carried out (0.8%, 1/114). Less than two thirds of the remaning 110 cytology reports (62,7%, 69/110) pathogenes were found via Gram Staining. In 41 cases (37.2%, 41/110), no pathogen was found.

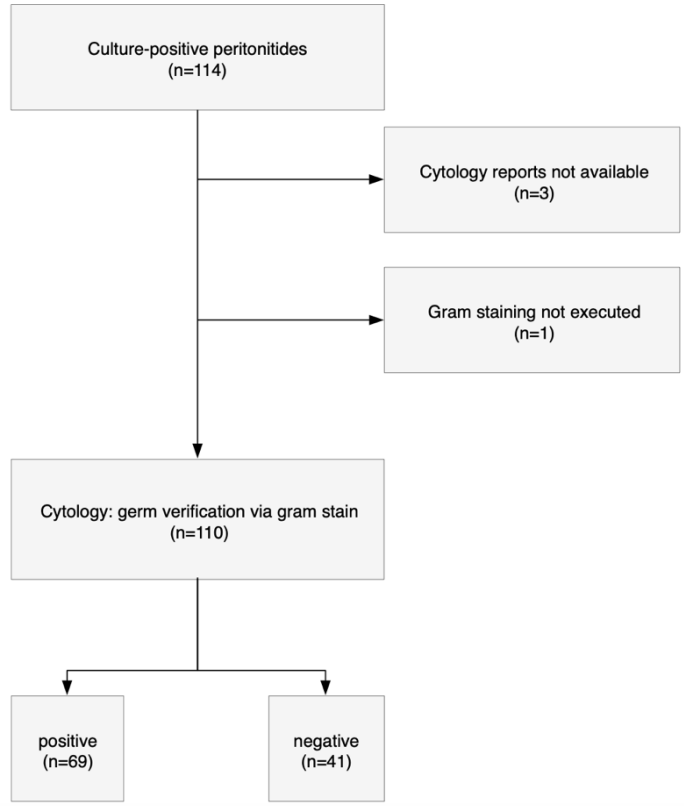

Figure 22: Flowchart of culture-positive peritonitis

As shown in Figure 23, the predominant causing organisms were found to be gram-positive cocci (84.1%, 58/69), followed by gram-negative rods (4.3%, 3/69), gram-positive rods (1.4%, 1/69) and gram-negative cocci (1.4%, 1/69) and fungal milieu (1,4%, 1/69). In 5 cases, the cytology showed more than one causing germ and was therefore polymicrobial (7.24% 5/69). Interestingly, in those 5 cases, was that in 3 episodes, cytology showed more organisms than the culture was able to detect. In one case of Staphylococcus Epidermis, the cytology showed gram-positive cocci and gram-negative rods, in one episode with Streptococcus agalactiae, cytology showed not only gram-positive cocci but also gram-negative cocci, gram-negative rods and gram-positive rods. One episode with Corynebacterium striatum was presenting as gram-positive cocci and gram-positive rods, while one infection was due to Streptococcus mitis and Staphylococcus aureus which presented as gram-positive cocci, gram-negative rods and gram-positive rods. In one case

the cytology showed a different result compared to the culture, namely an infection with Escherichia coli and Morganella morganii, which was described in cytology reports as gram-positive cocci and gram-positive rods.

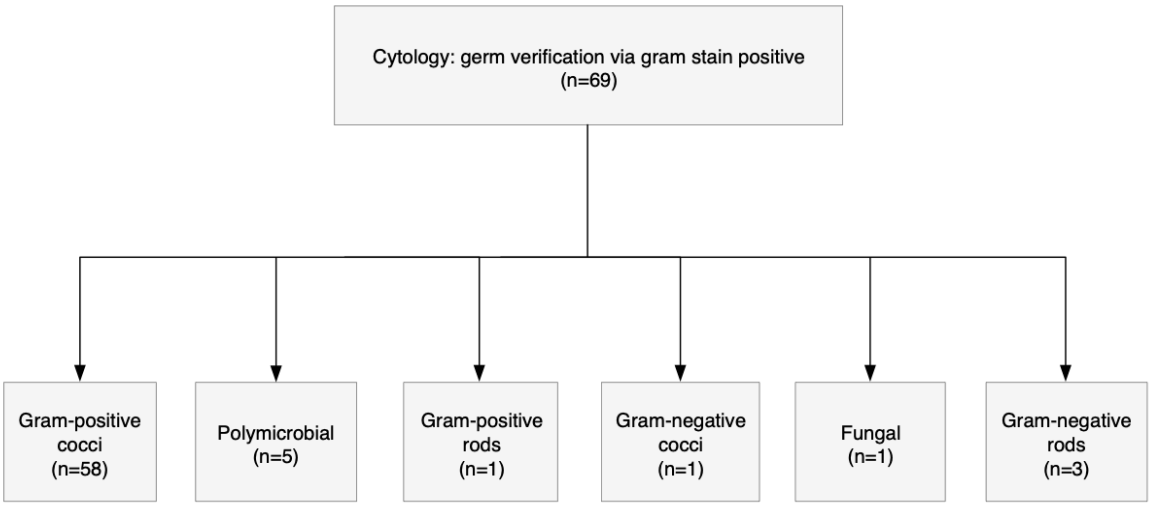

Figure 23: Flowchart of cytology in culture-positive peritonitis A

The verification of the causing organism was not positive in 41 CPP cases via the Gram's staining. These infections were most often caused by polymicrobial milieu (27%, 11/41), Staphylococcus epidermis (17%, 7/41), E. coli (12%, 5/41), S. aureus (10%, 4/41), Micrococcus spp. (5%, 2/41), Enterococci (5%, 2/41), Haemophilus influenzae (5%, 2/41), and each one of Staphylococcus caprae (2%, 1/41), Streptococcus sanguinis (2%, 1/41), Achromobacter dentrificans (2%, 1/41), Pasteurella sp. (2%, 1/41), Staphylococcus lugdenensis (2%, 1/41), Enterobacter sp. (2%, 1/41), Klebsiella oxytoca (2%, 1/41) and Coagulase-negative Staphylococci (2%, 1/41), which accounted for 16% of causing organism (see Figure 24).

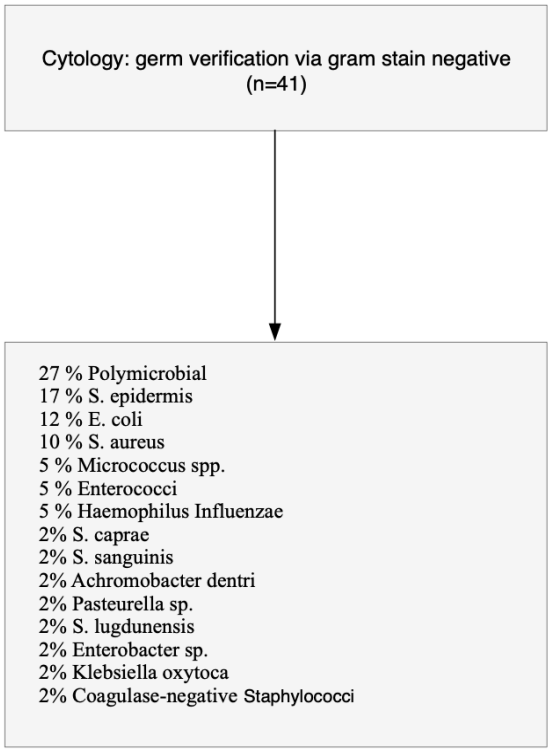

Figure 24: Flowchart of cytology in culture-positive peritonitis B

3.1.11.4 Outcome of CPP Episodes

As mentioned above, the number of leucocytes as well as the time until the number was in the normal range again, varied in the different entities. For further information please see chapter 3.1.9.1. In 114 episodes, 18 patients had to be treated temporarily with hemodialysis (15.7%).

3.1.12 Culture-negative peritonitis

Forty-one episodes of culture-negative peritonitis (CNP) (41/155, 26.4%) occurred in our patient cohort, thereby exceeding ISPD Guideline recommendations, see chapter 4.1.

Twenty-six CNP (26/41, 63.4%) occurred as first episode of a patients' history. Twelve (12/41, 29.3%) culture-negative episodes were found as a second episode of which 5 (5/41, 12.2%) were preceded also by CNP. Six patients (6/41, 14.6%) suffered 2 episodes of CNP. Two patients (2/41, 4.9%) were pretreated with antibiotics in other hospitals at time of applying at the outpatient department for Peritoneal Dialysis, 1 patient (1/41, 2.4%) was immunosuppressed and suffered from norovirus infection.

As shown in Figure 25, the absolute number of culture-negative episodes varied in the observed time period, with a peak in 2010 and lows in 2008 and 2014. When looking at the relative numbers compared with the number of absolute numbers per year, seen in Table 8, the year with the highest number of CNP, was also found to be in 2010 (44.4%), followed by 2015 (35.3%), 2017 (33.3%), 2007 (28.6%), 2008 (25.0%), 2009 and 2016 (each 21.7%), 2013 (21.4%), 2011 (20%), 2012 (16.7%) and 2014 (11.1%).

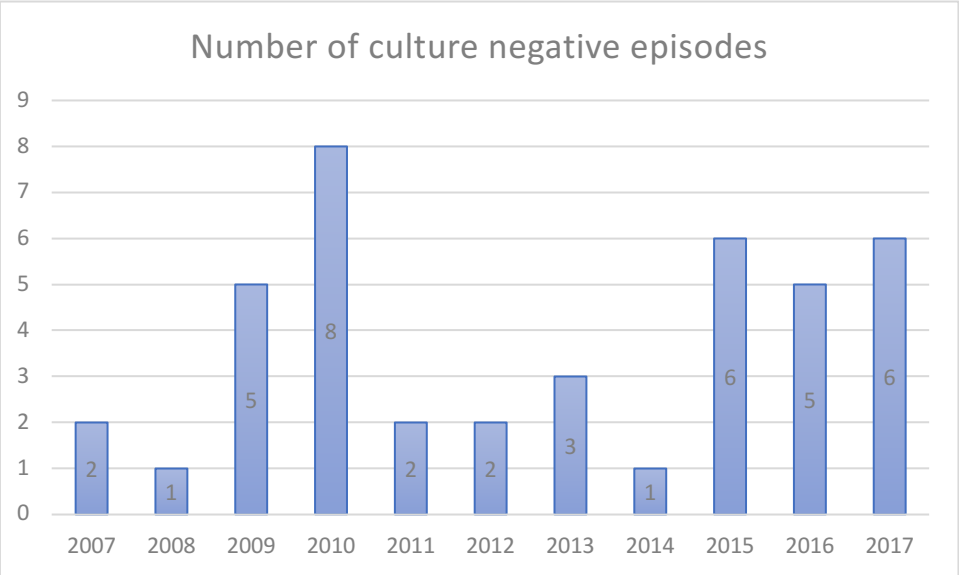

Figure 25: Absolute numbers of CNP

Year	Number of all peritonitides (n=155)	Number of culture-negative peritonitides (n=41)	Percentage
2007	7	2	*28,6%*
2008	4	1	*25,0%*
2009	23	5	*21,7%*
2010	18	8	*44,4%*
2011	10	2	*20,0%*
2012	12	2	*16,7%*
2013	14	3	*21,4%*
2014	9	1	*11,1%*
2015	17	6	*35,3%*
2016	23	5	*21,7%*
2017	18	6	*33,3%*

Table 8: Relative numbers of CNP

The suspected causes of CNP varied widely throughout the collective. Catheter related infections (4.87%, 2/41), concomitants by massive coprostasis (4.87%, 2/41), assumed reactions to plasticizer plastic of CAPD bag (4.87%, 2/41), dental focus (2.38%, 1/41), putrid tooth extraction (2.38%, 1/41), sigma diverticulitis (2.38%, 1/41), bacteremia (2.38%, 1/41), umbilical fistula (2.38%, 1/41), concomitant by gastroenteritis (2.38%, 1/41), lack of hygiene (2.38%, 1/41), concomitant after vessel surgery (2.38%, 1/41), subileus/ileus (2.38%, 1/41), concomitant after umbilical hernia operation (2.38%, 1/41)

were described in the reports but, most commonly, the reason for the infection remained unknown (58.53%, 24/41), see Figure 26.

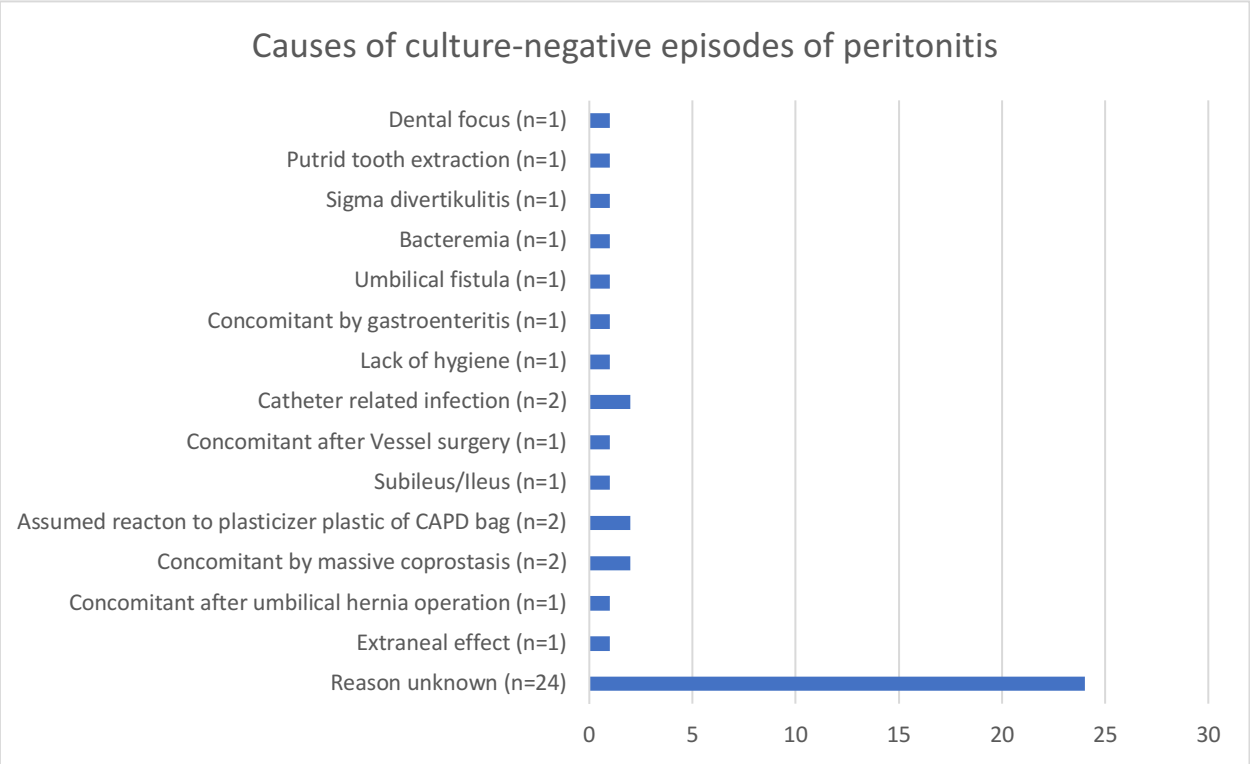

Figure 26: Causes of CNP

3.1.12.1 Cytology in culture-negative peritonitis

In cytology, we were able to distinguish between neutrophils and eosinophils using the Hemacolour staining technique, see chapter 2.4.1. Out of 41 culture-negative peritonitis, thirty-one (75.6%, 31/41) episodes showed predominant neutrophils in the dialysate effluent, while in 10 (24.4%, 10/41) episodes predominantly eosinophilic infiltration was assessed in the effluent cytology, see Figure 28.

In relation to overall peritonitis rate and CNP, in 2007, one neutrophilic (50%, 1/2) and one eosinophilic (50%, 1/2) episode occurred, while in 2008, one neutrophilic (100%, 1/1), in 2009, five neutrophilic (100%, 5/5), in 2010 3 eosinophilic (37.5%, 3/8) and 5 neutrophilic episodes (62.5%, 5/8), in 2011 one neutrophilic (50%, 1/1) and one eosinophilic episode (50%, 1/1), in 2012, two neutrophilic episodes (100%, 2/2), in 2013, two neutrophilic (66.6%, 2/3) and one eosinophilic episode (33%, 1/3), in 2014, one neutrophilic episode (100%, 1/1), in 2015, five neutrophilic (83.3%, 5/6) and one eosinophilic episode (16.6%, 1/6), in 2016, three neutrophilic (60%, 3/5) and two eosinophilic episodes (40%, 2/5) and

in 2017, five neutrophilic (83.3%, 5/6) and one eosinophilic episodes were causing the peritonitis. See Figure 27.

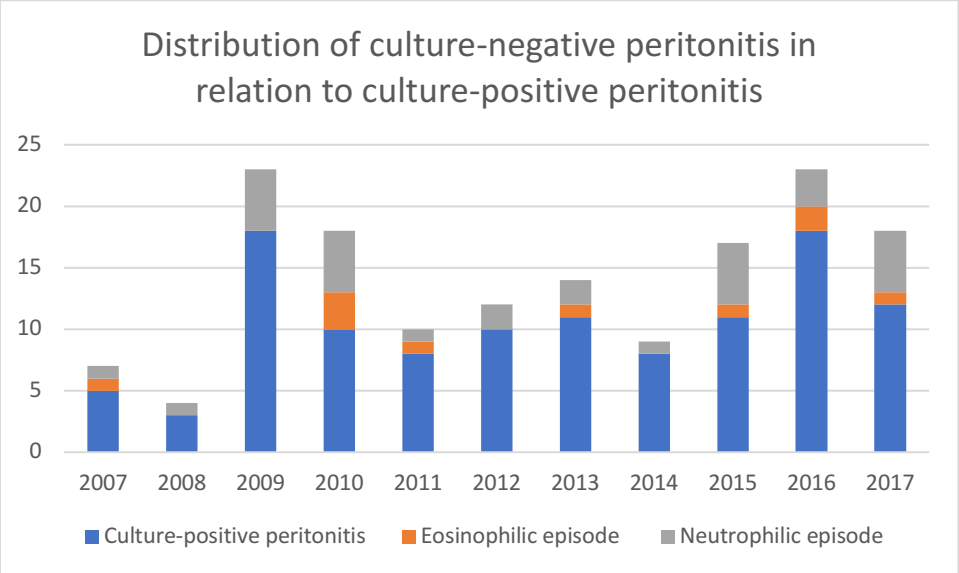

Figure 27: Distribution of CNP in relation to CPP

3.1.12.2 Neutrophilic CNP

Dialysate effluent cytology showed in 75.6% (31/41) of CNP a neutrophilic peritonitis. Out of these, the usage of Gram stain technique identified in 16 cases (51.6%, 16/31) possible pathogens, which were mainly gram-positive cocci (81.3%, 13/16), see Figure 28.

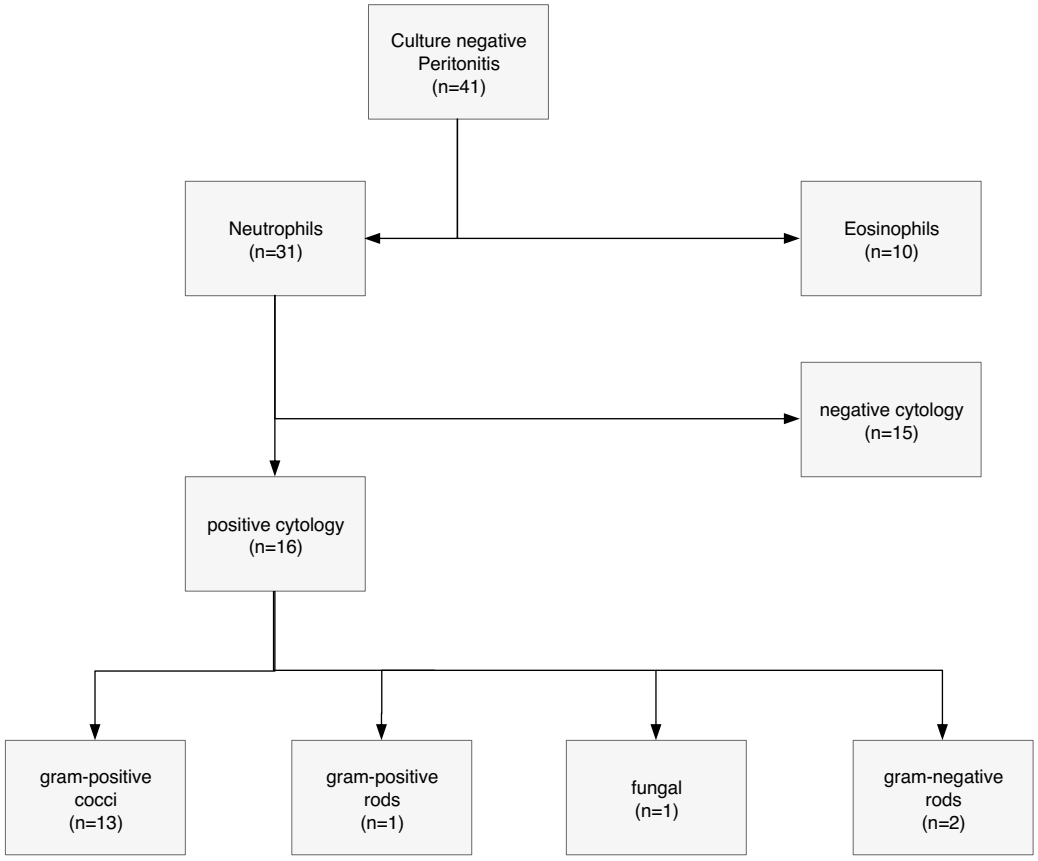

Figure 28: Flowchart of cytology in CNP

Out of 2 patients, who were treated in other hospitals with antibiotics, before sampling a culture and probably therefore leading to CNP, one patient showed gram-positive cocci in dialysate effluent cytology.

One episode out of 2 patients with CNP and marked coprostasis at the time of presentation to the hospital, was found to be caused by gram-positive cocci in the effluent cytology. Cytology was also able to identify a possible germ in patients with preileus/ileus (gram-positive coccus), after a surgery of an umbilical hernia (gram-negative and gram-positive rods, which is the reason for the number of 17 pathogens in 16 cases of positive cytology), bacteremia and putrid tooth extraction (both gram-positive cocci) and lack of hygiene (gram-negative rods).

	Positive cytology (n=16)	Negative Cytology (n=15)	p-value
Initial leucocytes/µl	2082.42	1541.53	*0.892*
Time until leucocytes <100/µl (d)	7,92	7,15	*0.545*
Switch to HD	18.7%	13.3%	*0.686*

Table 9: Presentation and outcome regarding cytology in CNP

As shown in Table 9, there was a difference between the groups of neutrophilic CNP regarding the cytology results, but statistically non-significant. In the group in which cytology showed no causing organism, initial leucocytes were lower (1541.53 leucocytes/µl vs. 2082.42 leucocytes/µl, p=0.892), time until leucocytes dropped below 100/µl was shorter (7.15 days vs. 7.92 days, p=0.545) and the transfer to hemodialysis was less frequent (13.3%, 2/15 vs. 18.7%, 3/16, p=0.686). Patients, in which cytology showed a germ, pain was less frequent compared to the group where cytology showed no germ (26%, 5/19 vs. 47% 7/15) while cloudy effluent was observed more often (37% 7/19 vs. 27% 4/15).

3.1.12.3 Eosinophilic CNP

For information of the entity of eosinophilic CNP and its diagnosis, see chapter 1.1.3.1.6.

In our study population, 10 episodes of eosinophilic peritonitis developed between 2007 and 2017, which were 24.4% (10/41) of the CNP or 6.4% (10/155) of all peritonitis episodes, respectively. Obviously, there was no eosinophilic peritonitis in CPP.
In only 4 patients, the data was assessable in matter of when patients first had complaints and these occurred, on average, one day before administration. Forty percent (4/10) described pain as the prevalent symptoms, while ten percent (1/10) reported cloudy effluent, ten percent (1/10) difficulties with the effluent, forty percent (4/10) nothing, respectively the data was missing. Two cases were thought to be due to the plasticizer in the plastic of the CAPD bag, 1 due to the Icodextrin-effect, 1 described as a concomitant peritonitis by massive coprostasis while the other causes remained unknown.

The average of initial leukocyte drainage in this subgroup of CNP was 568.22/µl ± 411.24 (range: 101 – 1163; 95% CI: 252.1160 – 884.3284), compared to 1820.7/µl ± 2407.67 (range: 106 – 9402; 95% CI: 937.5670 – 2703.8523; p=0.199) of the neutrophilic CNP group respectively, 1538.9/µl ± 2185.04 (range: 101 – 9402; 95% CI: 840.08 – 2237.71) of the culture-negative main group, and 6950,88/µl ± 11465.848 (range: 28 – 94300; 95% CI: 5113.3824 – 8788.38) for all PD patients with peritonitis.
The mean time until leukocytes count dropped below 100/µl was 6.5 ± 7.05 days (range: 1 – 20; 95% CI 0.61 – 12.39), compared to 7.53 ± 7.31 days (range: 1 – 30; 95% CI 4.58 – 10.49; p=0.403) in the neutrophilic CNP group, 7.29 ± 7.163 (range: 1 – 30; 95% CI 4.79 – 9.79) in the culture-negative main group, 7.12 ± 5.681 days (range 0 – 25; 95% CI: 5.95 –

8.29) in the culture-positive main group and 7.19 ± 6.07 (95% CI: 6.13270 – 8.24525, range 0 – 30) for all PD patients with peritonitis.
In three cases, no anti-infective therapy was administered, while 1 episode was treated with an antihistaminic agent, in 5 episodes the empirical antibiosis was continued and in one case we were not able to find any data regarding treatment. Regarding antibiosis also see: Chapter 5. Limitations.

3.2 Patients treated with ascites drainage

Three patients (2 male, 1 female) were treated in this time period solely with ascites drainage, using a PD catheter, supervised via the PD unit. Patients received minimal volumes of peritoneal dialysate installed inside the abdominal cavity for flushing the catheter periodically after ascites drainage. These amounts of dialysate are not contributing for any clearance. The frequency of ascites drainages depended on the amount of ascites produced and varied between daily drainages and drainages, once a week. The average age at ascites-drainage-start was 46.1 ± 13.62 (30.8 – 56.9). The underlying renal diseases were cardiorenal syndrome (2/3) and autosomal dominant polycystic kidney disease (1/3). The reason why the patient with ADPKD was treated with ascites drainages only, was because HD was the main therapeutic strategy and the PD catheter was only used for the ascites drainages, caused by portal hypertension, due to liver fibrosis. The mean time under drainage was 1.95 ± 1.36 (0.82 – 3.46) years.

In this cohort, 3 episodes of peritonitis occurred all within one patient and each episode was caused by E. coli, therefore effecting a centrum-specific rate of 0.512 episodes per year and a patient-specific rate of 1 episode every 23.41 patient months. The patient presented on average 2.66 ± 2.51 days (0 – 5) after first presentation of symptoms which were pain (n=3) and cloudy effluent (n=1).
The average initial effluent leucocytes were 4602.6/µl ± 816.8 (3909 – 5503) and the average time, until the dialysate leukocytes dropped was 11.6 days. Conscious, of the two entities, PD patients and patients with only ascites drainage are difficult to compare, PD patients showed a higher account of initial effluent leucocytes (6950,88/µl), but faster response (7.19 ± 6.07 days vs. 11.6 ± 9.6 days), when measured by amount of days until effluent leucocytes drop below 100/µl.

4 Discussion

The aim of this study was to evaluate the peritoneal dialysis program at the Medical University of Graz, Division of Nephrology including peritonitis rate and to find out whether dialysate effluent cytology has additional benefits for the diagnosis of a peritoneal dialysis associated peritonitis.

4.1 Peritonitis rate in comparison to the ISPD guidelines and ANZDATA

The ISPD guidelines recommend that peritonitis rate among PD patients should be less than 0.5 episodes per year at risk (11). At our center, we had an average of 0.234 episodes per patient year for PD-only patients and therefore match the ISPD recommendation. The mean time until leukocytes in the dialysate drop below 100/µl were 7.29 days in the overall cohort, 7.19 days in the PD-only cohort, respectively 6.41 days for the CPP gram-positive group, 8.35 days for the CPP gram-negative group, 13.25 days for the CPP with mixed germs, 7.29 days in the culture-negative group and 11.66 days in the ascites-only group. The ISPD guidelines recommend a target value of 5 days until peritoneal effluent should clear up, which happens earlier than normalization of cell count. The guidelines further recommend removing the catheter if there is no clinical improvement after 5 days of antibiotic treatment, but do not have any recommendations to remove the catheter regarding elevated levels of leucocytes. Interestingly, if gram-negative bacteria are involved, the dialysate takes longer to resolve. Patients treated only with ascites drainage, appeared to have regularly higher effluent cell count, often over 100 leucocytes per microliter, without showing any signs of peritonitis. In this patient population needs to be considered, that ascites cannot be compared to dialysate because of a different underlying disease with different pathomechanism and therefore incomparable composition of the fluid. As a consequence, there are other diagnostic criteria. Fever and abdominal pain are rare and patients usually present without any clinical characteristics, but a cell count of more than 250 granulocytes per liter or over 500 leucocytes per liter gives a lead to the diagnosis of spontaneous bacterial peritonitis, while the germ identification via cultures often remains negative (102).

We further compared our center-specific peritonitis rate with the rate reported by the ANZDATA register. This register includes 2440 PD patients in Australia and 823 PD patients in New Zealand between 2005 and 2015. Therefore, we could prove that our rate

is not only below the recommended by the ISPD guidelines, but also the ANZDATA (103).

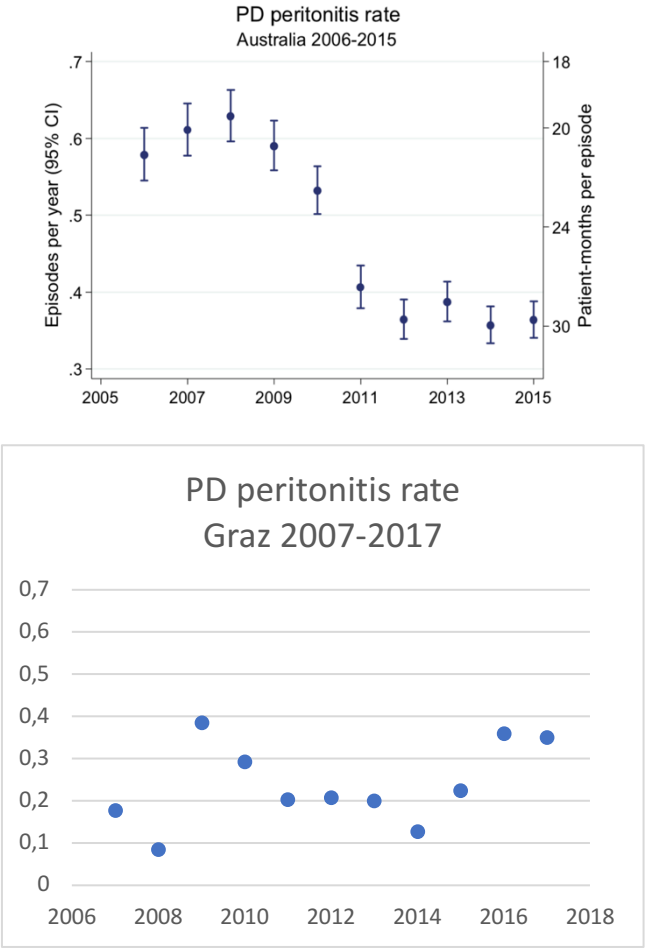

Figure 29: ANZDATA peritonitis rate vs Graz peritonitis rate

4.2 Improvement for cultivation standards

In total, 41 culture-negative episodes were observed in our cohort. The median annual rate of culture-negative peritonitis was 25.4 ± 8.9% (range: 11% – 44%). This exceeds the recommendations in the ISPD guidelines (target <15% CNP) and culture methods should be reviewed (11). In an electronic survey amongst microbiologist and senior PD nurses, conducted in the United Kingdom in 2016 in 53 centers, median the annual rate of culture-negative peritonitis was 15% (range 5 – 38%) (104). Worth mentioning, two episodes of CNP occurred after prior treatment at other hospitals before transferring to the PD department which altered the result and consequently, the centrum-specific rate, which has to be adjusted.

A possible explanation why our rate of CNP is higher than the median in the mentioned study above, is the technique of culture sampling. When patients arrived at the hospital

during the weekend, culture mediums had to be stored by room temperature or in the refrigerator and were kept there until the assay was performed, while empirical antibiosis had to be started right away. The effluent cytology and cultivation were first surveyed on the following working day. This exceeds the recommended time by the ISPD guidelines, in which the sample should be transported to the microbiological lab within 6 hours or at least incubated at 37 °C. Because of this study, we adapted the SOP at our hospital in order to further improve the diagnostic regime.

In some cases, dialysate effluent cell count and culture sampling were first performed on the first working day while already anti-infective treatment was given. This is supported by the fact that 26.8% (11/41) of CNP were found to occur on Mondays, also see chapter 3.1.7. Eleven CNP were "diagnosed" on Mondays because the culture was drawn and investigated on this day. Out of these 11 patients, 4 were already treated on the weekend with antibiotics, while the culture was drawn on Monday, which results in a delay in sampling of 24 hours in 3 cases and even 48 hours in one case.

A different way to store the cultures on the weekends could improve the rate of CNP in our center. Also, different caregiver drew different amount of effluent to the blood culture bottle, which may affect the result, although the guidelines recommend inoculating 5-10 ml respectively centrifuging 50 ml of effluent to reach sufficient sensitivity (11).

Regarding outcome and consequences, culture-negative and culture-positive peritonitis were compared and CNP were found to be more benign in the light of the fact, that the initial leucocytes were lower (1538.9/µl ± 2185.04 vs. 6950,88/µl ± 11465.848). When considering the time until normalization of the leucocytes (7.29 ± 7.163 for CNP vs. 7.19 ± 6.07 for CPP), CNP took slightly longer to resolve. Fahim et al. concluded that CNP is found to be less often complicated by hospitalization or catheter removal (12), which was not subject in our study. When distinguishing between eosinophil and neutrophil episodes, eosinophil group appeared with less inflammation measuring the initial leucocytes (568.22/µl ± 411.24 vs. 1820.7/µl ± 2407.67) and was found to resolve quicker (6.5 ± 7.05 days vs 7.53 ± 7.31).

As mentioned in chapter 1.1.3.1.2., not only centrifuging of 50mL effluent at 3.000 g for 15 minutes and with subsequent resuspension in the supernatant and inoculation on solid media, but also water lysis, Tween-80 blood agar and Triton-X-treatment are sensitive in diagnosis of peritonitis, which can help reduce rates of CNP.

4.3 Benefit of Gram stained effluent cytology

Out of 41 episodes of culture-negative peritonitis, cytology was able to distinguish 31 episodes of neutrophilic and 10 of eosinophilic peritonitis. Of 31 appearing neutrophilic episodes, in 51.61% of the cases, cytology revealed a plausible germ, which were in 81.25% gram-positive cocci. This finding is almost congruent with the relative number of gram-positive cocci in the cultured pathogens (84.1%, 58/69).

Hausmann et al. suggested, against, at that time valid guideline recommendation, to drop initial coverage of gram-negative bacteria when the bacteriologic cultures stay negative for forty-eight hours, since they rarely cause culture-negative peritonitis (101), see chapter 1.1.3.1.6. Our study showed, using the Gram's stain technique in effluent cytology, that gram-positive bacteria are indeed mainly responsible for this type of infection and it makes sense to initially start with antibiotic coverage for gram-positive pathogens, but in 3 cases (18.75%, 3/16) other pathogens were detected, hence also gram-negative coverage is necessary to eliminate all germs.

The reason why effluent cytology of patients treated with peritoneal dialysis is recommended in the ISPD guidelines is mainly based on one study, which stated that Gram's stain is a significant predictor for outcome of empirical antibiosis (105). In this Study by Lee et al., a cohort of 83 patients with 192 episodes of peritonitis between 1997 and 2006 were included, with 159 of them treated with initial empirical antibiotics, consisting of a first-generation cephalosporin and gentamicin. 64 out of 159 (40.3%) were cured by this treatment, while in 59.7% (95/159) this antibiotic regimen failed. In the subgroup, in which empirical treatment was successful, 15.6% of episodes (10/64) resulted in positive Gram's staining for bacteria, while in the empirical treatment failure subgroup, 40.0% (38/95) of Gram's stains for bacteria were positive and therefore the odds ratio for empirical treatment failure when Gram's stain for bacteria was positive, amounted to 3.60. Also, when sterile episodes were included, the OR summed up to 3.56, while the rate for positive Gram's staining was 30.2% (48/159). Since we could not completely assess the empirical treatment, we were not able to confirm this finding. This study by Lee et al. is limited by its design, since it's a retrospective analysis and also due to the antibiotic regimen which did not sufficiently cover MRSA and Enterococcus and therefore might alter the results since it is measured by resolution of infection owing to empirical antibiotic treatment. In our specific case, with a rather high rate of culture-negative episodes, Gram's Staining of effluent yielded in 51.6% of neutrophil CNP to identification of the germ species.

Another benefit, which has to be highlighted, is the faster result compared to a culture. Microbiological culture is obviously the gold-standard for identifying microorganisms, but if culture is not available, for example on weekends or because of anti-infective treatment initiation before diagnostic samples have been taken, effluent cytology may give a direction to the peritonitis origin.

Although, the main reason for the occurrence of the culture-negative peritonitis in our collective persisted indeterminate, gastrointestinal processes seem to play a major role. Seven episodes out of 17 affirmed causes were due to diverticulitis, umbilical fistula, gastroenteritis, subileus/ileus, coprostasis, or umbilical hernia surgery. This is concordant with the finding of Mishalov et al., which stated that constipation is an independent risk factor for peritonitis (44). Coprostasis was found to be the reason for one episode of culture-negative, cytology-positive peritonitis (gram-positive cocci) and for one episode of eosinophilic peritonitis. The patient with cytology positive result was suffering from an exit-site-infection with Staphylococcus aureus at the same time and recovered quickly after initiation with Vancomycin. Therefore, the cytology helped to detect the causing organism, although the microbiological culture was inconclusive. Since obstipation is a frequent condition in PD-patients, precautions should be taken.

We also evaluated the cytology results in culture-positive episodes and especially gram-positive cocci were found to be very often detected in the effluent cytology. When cytology showed a germ, the infection was mainly due to gram-positive cocci (84.1%, 58/69 in CPP and 81.3%, 13/16 in CNP) followed by polymicrobial milieu (7.24%, 5/69), gram-negative rods (4.3%, 3/69), gram-positive rods (1.4%, 1/69), gram-negative cocci (1.4%, 1/69) and one fungal episode (1.4%, 1/69).

Another interesting aspect, which needs to be highlighted, is the discrepancy between culture and cytology as mentioned in chapter 3.1.11.3. In polymicrobial milieu, cytology revealed more germs than cultured, while at the same time, cytology's weakest point was in CPP with polymicrobial milieu.

In our cohort, 62.7% (69/110) of culture-positive peritonitis could also be identified with Gram's staining but 37.3% (41/110) remained undetected. One possible explanation why there are lots of negative results in the Gram's staining of direct effluent cytology might be the dependency of the examiner. If the analysist is not as advanced as others, the rate of negative results may increase. Furthermore, the result of cytology depends on the sample material. As happened in one case of the effluent, when the liquid's density is too high, a

proper examination cannot be performed. Compared to other recent published studies, Gram's Stains yielded in high positive results. A recently, in 2019, published paper investigated the performance of Gram's stains compared to culture methods. Among other parameters, they examined dialysate fluids of patients, who felt clinically to suffer from peritonitis and came to the result that bedside inoculated blood culture bottle kits had a positivity rate of 92.8% compared to Gram's stain with only 7.7% and every time Gram's stain showed a result, so did the culture. As a consequence, they suggest that routine Gram's staining is not necessary, especially in consideration of the time needed to perform these laboratory procedures (106). This is in contrast to our findings, since we found rates of positive Gram's stains among culture-negative peritonitis of 51.6% and 62.7% among culture-positive peritonitis, similar to Rathore et al. with 52.4% (107). This may be due to different investigation methods, since Buchanan et al. used 30cells/mL as minimum cut-off value for diagnostic, other than recommended in the ISPD guidelines, and therefore perform more often Gram's stains. Due to the lack of reported information of the centre-specific staining standards, there is no explanation for this variance of positivity in Gram's staining.

On the other hand, but not completely commensurable, they seem to have a well-functioning process of culture sampling with not only direct inoculation of blood culture bottles by enrichment through centrifugation and inoculation of the precipitate into pediatric blood culture bottles (BacT/ALERT PF Plus, Biomerieux), but also inoculating directly onto solid media (Columbia Blood and Chocolate Agars, Oxoid, Thermoscientific, Basingstoke, UK) in aerobic environment for 48 hours (106).

When comparing culture-negative peritonitis regarding cytology behavior, it becomes clear that episodes with a germ detected by a cytological procedure, respectively Gram's stain and Hemacolour staining, have higher leucocytes, longer time until relieve and a higher percentage of patients who have to switch to hemodialysis, even though the p-values are not significant probably due to small numbers of this specific patient collective. This still is a measurably impact of cytological analysis, which can benefit the patient in terms of even more urgent antibiotic treatment when leucocytes are at high levels.

In any case, awareness needs to be increased, that diagnostic samples must be gathered before any anti-infective treatment and before starting therapeutic high frequent cycles with short dwelling times to minimize peritonitis induced abdominal pain.

4.4 Benefit of Hemacolor stained effluent cytology

Effluent cytology also does have predictive value since eosinophilic episodes have a better outcome than other culture-negative episodes and may have a non-infective origin to examine for.

4.4.1 Eosinophilic episodes

Eosinophilic episodes were found to be associated with a less strong enhancement of leukocytes compared to culture-negative episodes and normal episodes, and also observed to be faster regarding leukocytes to drop below 100 /µl. The average amount of leucocytes in eosinophilic episodes (568.22/µl) was found to be significantly lower, compared to the main group culture-negative (1538.9/µl) and the overall PD cohort (6950.88/ µl). The mean time until leukocytes count dropped below 100/µl was 6.5 ± 7.05 days compared to 7.53 ± 7.31 days in the neutrophilic CNP group, 7.29 ± 7.163 in the culture-negative main group, 7.12 ± 5.681 days in the culture-positive main group and 7.19 ± 6.07 for all PD patients with peritonitis.

One patient, later diagnosed with peritonitis, complained about obstipation and pain, probably due to extensive consummation of Cocoa powder. Therefore, he was treated with laxatives. Because of the serious pain in the lower abdomen, abdominal and pelvic CT was realized. At the same time, he developed a common cold with rhinorrhea and unproductive cough. After application of laxatives, the patient presented with watery diarrhea. Because of suspected peritonitis, the patient started inpatient treatment while the effluent was at first presenting only with some eosinophils, but during the course of inpatient care, including gastroscopy and colonoscopy, the diagnosis eosinophilic peritonitis was established due to highly elevated leucocytes with lots of eosinophils and pain as the second column of diagnosis. Back then, this eosinophilic peritonitis was traced back as a collateral reaction to obstipation followed by diarrhea. The patient received Vancomycin and Ceftazidime one single time, no antihistaminic treatment and because of the uncomplicated process, the patient was discharged shortly after.

One patient, later diagnosed with eosinophilic peritonitis, came already 4 days in advance to the PD unit because of pain but the effluent was ordinary with normal cell count and normal Gram's stain and the catheter presented in a normal position in the abdominal x-ray. Four days later he presented to the PD unit again and the cell count was over 200 with mainly eosinophils. Empirical antibiosis was administered and the patient could be

released 5 days later. The reason remained uncertain. Interestingly, relative eosinophilic cells were elevated in differential hemogram (8% compared to normal values of 5%), while normal absolute eosinophils. Diagnosis of peritonitis was made because of elevated leucocytes and symptoms. The cultures remained sterile and antibiosis was stopped after 1 week.

One patient was transferred to our unit due to exsiccosis, which suddenly developed 2 days before administration. She was rehydrated with parenteral volume substitution and her diuretics were discontinued. Shortly after, hyperphosphatemia developed, which was treated with Sevelamer Carbonate and in the course of in-patient stay, eosinophilic peritonitis developed. Single loading dose of Vancomycin was administered, and patient recovered quickly.

One patient complained about pain in the lower abdomen when draining the cavity, which improved over night and only discrete pain remained in the morning. Nevertheless, the patient presented at the department in the morning to clarify. Dialysate was really viscous so that a cell count was initially not possible, but microscopy showed lots of erythrocytes and signs of peritonitis, while Gram's stain showed no result. Because of hardly any symptoms, no therapy was administered, and the patient was sent home with follow-up appointments. Later on, lots of eosinophilic granulocytes could be observed and reaction to the catheter or to the solution was suspected, but after only a few days symptoms disappeared and effluent was normal again. Two weeks later, patient presented again with abdominal pain and results in microscopy were the same as last time. Because the pain was mainly during the drainage of the solution, doctors then supposed that undertow might cause the pain. Therefore, the effluent velocity during APD was slowed down and the patient was not raising any complaints anymore and eosinophils were regredient.

This episode occurred 52 days after implantation of the PD catheter. The type of peritoneal dialysis was adapted from APD/CCPD to CAPD and shortly after that, the pain decreased and led to normalization of cell count. Another case of close-to-implant eosinophilic peritonitis happened in another patient 19 days after implantation and during the in-patient enrollment. Eosinophilic peritonitis is known to establish shortly after PD catheter insertion, but may be less common when using a percutaneous catheter placement technique, which has to be evaluated in future studies, examining a large cohort of patients (108).

One case of eosinophilic peritonitis occurred in a patient, who was treated with antibiotics 2 weeks prior because of an ESI. At time of presentation, the patient complained about

abdominal pain and irregular stool. The working diagnosis as an infection with Clostridium difficile could not be proven in the stool diagnostic methods. The cell count showed clearly elevated leucocytes of 623/µl with elevated eosinophils, and slightly elevated eosinophils in the hemogram (7%). Vancomycin and Ceftazidime was administered, and symptoms disappeared quickly. The chest radiography showed findings consistent with Chilaiditi's syndrome which is a rare dislocation of intestine between liver and the diaphragm which is, in most of the cases, asymptomatic, but can lead to abdominal symptoms (109). Whether this if a risk factor for eosinophil peritonitis is, as far as we know, not reported in literature.

In one case of eosinophil peritonitis, the PD unit team was able to find out that the patient was not doing PD adequately, due to technical problem since at least one week. 200ml of dialysate was therefore left in the abdominal cavity from that time on. Signs of peritonitis with blood coagulum in cytology, combined with elevated leucocytes, respectively eosinophils, and pain led to the diagnosis. Furthermore, the patient was compromised with diarrhea caused by norovirus. After recovery, the therapy continued with IPD at the centre. Eosinophilic episodes usually are linked to a more benign outcome (94) and are also affected by co-factors such as high IgE levels, Vancomycin administration, and allergic reactions to parts of the PD system (93, 95). They also show lower initial leucocytes and are therefore may partly be a different entity of peritoneal dialysis associated peritonitis. Despite that, the main reason for eosinophilic episodes remained unclear.

4.5 Comparison of symptoms of culture-positive peritonitis and culture-negative peritonitis

Our survey showed that patients with culture-negative peritonitis reported less often pain (32.0% vs 45.7%) and also had less often cloudy effluent (24% vs 29.1%) due to fewer cells in the cell count. Unfortunately, the lack of data was greater in patients who suffered from CNP (26% vs 5.9%). CNP are known to be more benign when it comes to outcome and are likely to be associated with previous antibiotic treatment (12). The previous antibiotic treatment might explain less symptoms and cloudy effluent, because of a previous medicated infection which also targeted the peritoneal germ but not completely wiped it out. In our cohort this might be an explanation for 2 CNP episodes.

Furthermore, there were also different results regarding initial leucocytes, days until normalization of the leucocytes, symptom pain and switch to HD, also see chapter 3.1.10.2.

4.6 Fungal prophylaxis

Although, fungal peritonitis is associated with higher rates of mortality and transfer to hemodialysis, there are contrary statements considering fungal prophylaxis during an antibiotic treatment in order to prevent fungal peritonitis and is therefore still one of the most discussed topics in peritoneal dialysis anti-infective prevention and treatment. In the ISPD Guidelines of 2016, it is recommended to use antifungal medication when PD patients receive antibiotic treatment due to any reason (11). This is based on two randomized controlled studies, which reported a significant reduction of fungal peritonitis, using either oral nystatin (110) or fluconazole (111). One of these two studies was a randomized prospective study, carried out in Hong Kong, China in 1996, including 397 patients, in which the cohort was split in two groups, whereby in one group, oral nystatin was administered, whenever antibiotics were prescribed, regardless the indication. They found, that the probability of Candida-peritonitis-free survival after two years was significantly higher in the group, which received nystatin as prophylaxis, compared to the other group (0.974 vs. 0.915; $p<0.05$). In this study, forty-four percent of candida related peritonitis occurred without prior antibiotic treatment in the past 3 months, whereas the authors state, that they conclude an overall reduction of this organism rather than a reduction in antibiotic related infection. Furthermore, they could not unambiguously exclude that patients did not use antibiotics by themselves without accounting in the study, since this is a common given situation in this area and candida related peritonitis was not completely preventable with oral nystatin (110). The second one was made in Manizales, Colombia in 2010, which was also designed as a prospective, randomized trial. They included 434 episodes including 402 bacterial causes and 32 mycotic causes, of which only 18 were preceded by antibiotic treatment. Three episodes of fungal peritonitis occurred in the group with prophylactic fluconazole administration and 15 episodes happened to be in the group without prophylactic treatment (111).

In our collective 3 other patients were diagnosed with fungal peritonitis. In the total observational period, only 2 patients received fungal prophylaxis, while one of these patients, who received prophylactic antimycotics, was suspected to have an infection with fungi, but fungal infection was neither confirmed, nor the catheter removed, which must

ensue in case of this type of infection. The decision to give routinely antimycotics whenever using intraperitoneal antibiotics, should be considered, factoring in demographic and geographic aspects. At our center, we refuse to administer fluconazole or nystatin as a matter of routine, especially due to side effects and undesired medication interaction, based on our center-specific low incidence.

4.7 Seasonal distribution of peritonitis

The distribution of peritonitis per month was interestingly accompanied with a peak in July and downs in June and August. A reason why this happened, could be that, at the beginning of summer in July in Austria, due to climate change and heat, patients tend to sweat more, and this may cause hygiene deficits, which affect quality of care in peritoneal dialysis. Earlier studies performed by Kim et al. and Szeto et al. showed a significant increase of infections in months with higher temperature and humidity (112, 113), but of course, were done in countries with a different climate, compared to Austria. Newer studies reported not an overall increase of infections, but a different distribution of germs throughout the year. Spring and summer are dominated by coagulase-negative staphylococci, summer and autumn by gram-negative pathogens and in the winter season mainly corynebacterial (114-116).

In our patient cohort we partly detected a similar trend. We indeed identified CNS in spring and summer and no CNS in autumn, but they also occurred in our population in the winter season. The only case Corynebacterium was detected, happened in winter but this may also be accidentally. A possible explanation why we found different results may be because of the different geographic areas and environment and therefore germs.

Patient's awareness should be increased to intensify their basic hygienic actions, changes of catheter exit side bandage replacement and adherence for avoiding swimming in inappropriate environment.

Interestingly, we found a difference of the occurrence of CNP regarding seasons. In winter, twenty-three % of peritonitis were found to be CNP, in spring 19%, in summer 18% and in autumn 47%. As far as we know, there have not been reports about significant differences in CNP regarding seasons in the literature. One possible explanation of this circumstance might be, that in this season a different organism, which is difficult to grow and therefore hardly identifiable, is accountable.

4.8 Other findings

In our cohort, peritonitis occurred in 56.96% (90/155) in APD/CCPD treatment and in 39.87% (63/155) in CAPD, but mainly because APD/CCPD was the more frequent treatment in our cohort during the observational period. If peritonitis episodes are normalized on patients' risk under treatment, relative occurrence was similar showing a center specific peritonitis rate in APD/CCPD patients of 0.2518 per year compared to 0.2238 per year in CAPD patients. Due to our data and other study results the type of PD technique, whether APD/CCPD or CAPD, does not seem to influence the incidence peritonitis rate (64-69).

Noteworthy, patients reported that first symptoms occurred averagely 1.34 days before administration, varying insignificantly between 1.62 days for APD and 0.871 days for CAPD (p=0.208), despite patients are strictly advised to report at the department by first appearance. This may suggest, that patients need even more emphatic education of the exceptional urgency of timely administration. Patients, performing APD, usually see their dialysate effluent only twice a day, videlicet in the morning after a lot of exchanges during the night, and in the evening after a long dwell time during the days, while patients using CAPD see their dialysate effluent due to multiple exchanges throughout the day, ordinarily more often and may therefore react more quickly to cloudy effluent.

The only parameter which seemed to affect the peritonitis rate statistically significant in our study was time under PD. Peritonitis-free patients had undergone a mean PD duration of 26.3 ± 18.5 months, while patients, suffered from peritonitis, had undergone a mean PD duration of 40.9 ± 26.3 months (p=0.000). Krishnan et al. and Perez Fontan et al. reported about negative effects of PD duration on mortality rate (OR 1.02/month, p=0.02) and non-resolution of peritonitis, defined either as death of the patient caused by peritonitis, catheter removal or switch to HD (49, 58). In the study carried out by Krishnan et al, patients who had undergone PD for more than 2.4 years, the nonresolution rate has been 24.4% compared to 16.5% for patients who had been treated with PD less than 2.4 years (p=0.05) (58).

Two culture-negative episodes occurred in a patient, diagnosed with encapsulating peritoneal sclerosis (EPS) on a subsequent date. This adverse outcome of peritoneal dialysis is extremely seldom but associated with around 50% mortality within 12 months of diagnosis. There is no evidence for strategies to prevent EPS, but reducing dialysate

glucose exposure, preventing peritonitis and usage of pH-neutral, low glucose degradation product dialysis solutions seem to positively affect the appearance (117).

Regarding diabetes mellitus, we discovered that especially in the first year after PD-start, the percentage of patients who are peritonitis-free are lower than in the collective of patients who do not suffer from this underlying renal disease (88.7% vs 77.6%) which may suggest, that these patients should be treated and educated even more carefully, particularly in this first year. The reason of this cognition might be because patients with diabetes mellitus are related to a higher transport rate compared to patients without this kind of disease (118), but apparently high peritoneal transport type in diabetic PD patients is not associated with all-cause mortality (119). Another explanation could be the greater non-adherence of patients or worse condition of the immune defense. In patients with diabetes mellitus, the mitogen-activated protein (MAP) kinase pathway is activated as well as transcription factors like nuclear factor 'kappa-light-chain-enhancer' of activated B-cells (NF-κB), which may be related to complications like nephropathy, impaired healing or atherosclerosis, but also pro-inflammatory cytokines, for example TNF-alpha, Interleukin-6 or Interleukin-1beta, are found to be elevated in diabetes, which further compromises patients' immune system's ability to cope with infections (120). Although diabetes mellitus has been described in the past as risk factor itself, in our study cohort, the percentage of PD patients with diabetic nephropathy, who had at least one episode of peritonitis (37.2%, 16/43), did not exceed the non-diabetic study population (38.2%, 79/207) ($p= 0.907$).

Also, regarding underlying renal disease, in patients with ADPKD autosomal polycystic kidney disease, peritonitis was more common than in patients without this morbidity (47% vs. 37.2%, $p=0.766$). Two recently published studies by Boonpheng et al. and Sigogne et al. reported about no negative impact of ADPKD on incidence of peritonitis (121, 122). Boonpheng et al. conducted a meta-analysis, including 14673 patients and came to the result that neither the risk of technique failure, nor the risk of peritonitis is increased in patients suffering from ADPKD (121). As far as we could assess, there is no association between IgA nephropathy and thrombotic microangiopathy related to elevated risk for this type of infection reported in literature.

The fact that some patients suffered from 5 or 10 episodes of peritonitis while others did not or only once, underlines the importance of different habits and manners, whereby a

questionnaire might be a useful tool to evaluate these. This could lead to improvement of training courses.

4.9 Preanalytical discrepancies

Within the study we found some inconsistent approach in the preanalytical procedure of our PD unit. Firstly, the amount of effluent drawn into the rapid blood culture bottle kits was varying, depending on the caregiver. Also, the time until the sample reached the laboratory was exceeding the recommended time period of a maximum of 6 hours (11), which may affect the cultural rate and may therefore be partly responsible for the high rates of culture-negative results in our center, which, based on this observation, showed the need to improve effluent cultivation standards further, which will be the next step of improving therapeutic strategies at our center.

5 Limitations of our study

Even though we evaluated a time period of 11 years, patients were all treated in a single center, therefore may contain bias due to standards in selection of patients, diagnostics and treatment. Multicenter evaluation might provide more information about regional and geographical differences and increase total number of evaluable episodes.

Mainly due to the retrospective analysis, data quality was partly incomplete, especially a lack of standardized documentation of possible underlying infection origin, catheter associated infections or exit site infections. There was a discrepancy of classification of number of documented relapses in the doctor's letters und factual events, probably due to non-compliance of the Guideline's definitions.

Antibiotic and other anti-infective treatment is partly documented handwritten and archived, so due to this lack of data in our MEDOCS system, we did not evaluate this data.

Due to the retrospective design, not all risk factors, mentioned in the introduction, such as comorbidities which may change during the patients' lifetime or intraperitoneal pressure, which has not been evaluated in the past at our center, were object of investigation in our study and are therefore part of the limitations. Furthermore, our department uses only the Tenckhoff catheter, consequentially we were not able to investigate if peritonitis rates are varying among the different designs, but which is, on the other hand, also an advantage to rule out this bias.

Even the limitations do not affect the aim of the study, which was to investigate the benefit of cytology.

6 Conclusion

Peritonitis is such an important complication of PD, since it is a major cause for treatment failure and deaths among those patients, hence, prevention and timely treatment are crucial. However, microbiological diagnosis, which is usually performed via effluent cultures, takes some time and therefore Gram's stains and Hemacolor staining might be a useful and additive feature.

We conducted a retrospective single center analysis and included all patients, who underwent any type of PD between 01.01.2007 and 31.12.2017 at the Clinical Division of Nephrology at the University Hospital in Graz. Patient's history was searched for elevated leucocytes in their peritoneal dialysate effluent and linked with doctors' letters. Microbiological analysis, which includes at our center automated cell count, Hemacolor and Gram's staining, microscopic assessment of the effluent sediment, was evaluated regarding benefit and compared to the results of the effluent culture.

In total, 253 patients were included, of which 250 performed only PD and were therefore investigated thoroughly. The mean age at the start of PD was 56 ± 15 years among the 68% of men and 32% of women, who suffered 155 episodes of this type of infection in 662.7 years at risk (centre-referential- rate of 0.2334). In 52%, Gram's stain was able to detect a possible germ in CNP episodes, while in only 63% of CPP. Ten out of 41 CNP showed mainly eosinophilic granulocytes in the effluent, an entity of peritonitis with other characteristics compared to neutrophilic CNP or CPP, such as initial leucocytes at diagnosis (568/µl vs. 1820/µl and 8346/µl) or switch to HD (0% vs. 16% vs. 16%). When comparing CNP with positive Gram's cytology with CNP with negative Gram's cytology, it appears that the former are associated with higher initial leucocytes (2082/µl vs. 1541/µl, longer time until leucocytes return to normal values (7.92 days vs. 7.15 days) and are more frequent related to switch to HD (18.7% vs. 13.3%).

The PD center in Graz not only matches the standards of the ISPD guidelines, but also have similar, if not better, rates to those reported in the ANZDATA register. Behind that, we did find a rather high annual rate of CNP at our center, which has to be improved. Cytology benefits the therapy of CNP, because it showed in 52% of neutrophilic CNP a possible germ in Gram's stained effluent and further, distinguished those from eosinophilic episodes in Hemacolor stained effluent, which need different or even no treatment at all. Also, effluent cytology has a predictive value since the varying outcome of neutrophilic

CNP, eosinophilic CNP and CPP. Since gastrointestinal issues are concomitant factors, the sensitivity and awareness should be raised for these kinds of illnesses and treated preventively. Because of the peak of infections in July, there should be more trainings and re-education before months with higher temperature and humidity.

For the improvement of the cultural rate not only the pre-analytic procedure, but also the cultural method (i.e. broth inoculation method) should be evaluated by the local microbiological institute. The broth inoculation method is one proposal recommended by the guidelines and could further reduce the cultural negative rates in this center (21).

In this department, the center-specific pathway, how the guideline is implemented via a local “standard operating procedure”, should be evaluated, tools like a standardized patient questionnaire supplemented and adherence increased by a training course for the responsible caregivers.

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