Neutrophil function in patients with chronic kidney disease: a systematic review and meta-analysis

Von der Medizinischen Fakultät
der Rheinisch-Westfälischen Technischen Hochschule Aachen
zur Erlangung des akademischen Grades
einer Doktorin der Medizin
genehmigte Dissertation

vorgelegt von

Jane Sophie Lauxen

aus

Koblenz

Berichter: Priv.-Doz. Dr. Heidi Noels, Ph.D.
Univ.-Prof. Dr. rer. nat. Claudia Göttsch

Tag der mündlichen Prüfung : 01 . 06 . 2026

Diese Dissertation ist auf den Internetseiten der Universitätsbibliothek online verfügbar.

Für meinen Ehemann Alexander, der mit der größten Geduld jede Träne trocknet, jedes technische Problem beseitigt und mehr an mich glaubt als ich es je könnte.

Publikation, die die Dissertation darstellt:

Lauxen, J.S., Vondenhoff, S., Junho, C.V.C., Martin, P., Fleig, S., Schütt, K., Schulze-Späte, U., Soehnlein, O., Prates-Roma, L., Döring, Y., Baaten, C.C.F.M.J. and Noels, H. (2025), Neutrophil Function in Patients With Chronic Kidney Disease: A Systematic Review and Meta-Analysis. Acta Physiol, 241: e70057. https://doi.org/10.1111/apha.70057

Check for updates

Acta Physiologica

RESEARCH PAPER OPEN ACCESS

Neutrophil Function in Patients With Chronic Kidney Disease: A Systematic Review and Meta-Analysis

Jane Sophie Lauxen[1] | Sonja Vondenhoff[1] | Carolina Victoria Cruz Junho[1] | Philipp Martin[1] | Susanne Fleig[2] | Katharina Schütt[3] | Ulrike Schulze-Späte[4] | Oliver Soehnlein[5] | Leticia Prates-Roma[6] | Yvonne Döring[7,8,9,10] | Constance C. F. M. J. Baaten[1,11,12] | Heidi Noels[1,11,12]

[1]Institute for Molecular Cardiovascular Research (IMCAR), RWTH Aachen University, Aachen, Germany | [2]Department of Nephrology and Clinical Immunology, RWTH Aachen University, Aachen, Germany | [3]Department of Internal Medicine I—Cardiology, Angiology and Internal Intensive Care Medicine, RWTH Aachen University Hospital, Aachen, Germany | [4]Section of Geriodontics, Department of Conservative Dentistry and Periodontics, University Hospital Jena, Jena, Germany | [5]Institute of Experimental Pathology (ExPat), Center for Molecular Biology of Inflammation (ZMBE), University Hospital Münster, University of Münster, Münster, Germany | [6]Biophysics, Center for Integrative Physiology and Molecular Medicine (CIPMM), Center for Human and Molecular Medicine (ZHMB), Center for Gender-Specific Biology and Medicine (CGBM), Faculty of Medicine, Saarland University, Homburg, Germany | [7]Division of Angiology, Swiss Cardiovascular Center, Inselspital, Bern University Hospital, University of Bern, Bern, Switzerland | [8]Department for BioMedical Research (DBMR), University of Bern, Bern, Switzerland | [9]DZHK (German Centre for Cardiovascular Research), Partner Site Munich Heart Alliance, Munich, Germany | [10]Institute for Cardiovascular Prevention (IPEK), Ludwig-Maximilians-University Munich (LMU), Munich, Germany | [11]Department of Biochemistry, CARIM, Cardiovascular Research Institute Maastricht, Maastricht University, Maastricht, the Netherlands | [12]Aachen-Maastricht Institute for Cardiorenal Disease (AMICARE), University Hospital RWTH Aachen, Aachen, Germany

Correspondence: Heidi Noels (hnoels@ukaachen.de)

Received: 8 December 2024 | **Revised:** 1 April 2025 | **Accepted:** 3 May 2025

Funding: This work was supported by the German Research Foundation (DFG) Project-ID 322900939—SFB/TRR219 [M05 to H.N., C07 to K.S., C09 to L.P.-R.], Project-ID 403224013—SFB 1382 [A04 to H.N.], the DZHK (German Centre for Cardiovascular Research) [B23Ex to H.N. and Y.D.], the Alexander von Humboldt Foundation [postdoctoral fellowship to C.V.C.J.], the RWTH Aachen, Start-up Funding [StUpPD_436–23 to C.V.C.J.], the Interdisciplinary Centre for Clinical Research, Faculty of Medicine, RWTH Aachen University [PTD 1-12 to H.N.], and the Dutch Heart Foundation [2020T020 to C.C.F.M.J.B.] as well as the Deutsche Stiftung für Herzforschung [F/61/23 to C.B.]. O.S. receives funding from the DFG (TRR332 projects A2 & Z1, CRC1123 project A6, CRU342 project P1, SO876/16-1). Also, this work supported by EFSD on "Multi-System Challenges in Diabetes, Obesity and Cardiometabolic Disease" 2024 (to H.N.). The funding sources were not involved in the conduct of research and/or data interpretation.

Keywords: chronic kidney disease | neutrophils | reactive oxygen species

ABSTRACT

Background: Patients with chronic kidney disease (CKD) are at increased cardiovascular risk. Since neutrophils play a central role in atherosclerosis and cardiovascular disease, this study analyzed neutrophil function in CKD patients.

Methods: A systematic review of neutrophil function in CKD patients compared to controls was performed according to PRISMA guidelines by searching PubMed and the Web of Science. A meta-analysis summarized the production of reactive oxygen species (ROS) in CKD patients on dialysis in Forest plots. Influencer outlier analyses evaluated risk of bias.

Results: Overall, 92 studies were included, of which 18 in the meta-analysis. Although study heterogeneity was high, the systematic review identified primarily reduced phagocytosis capacity but increased neutrophil degranulation and basal ROS production in neutrophils from CKD patients on hemodialysis compared to controls. Phagocytosis and basal ROS production were

Abbreviations: CI, confidence interval; CKD, chronic kidney disease; CVD, cardiovascular disease; fMLP, N-formylmethionyl-leucyl-phenylalanine (officially called fMLF); HD, hemodialysis; MPO, myeloperoxidase; NETs, neutrophil extracellular traps; NOX, NADPH-oxidase; PD, peritoneal dialysis; PMA, Phorbol 12-myristate 13-acetate; ROS, reactive oxygen species; TNF, tumor necrosis factor.

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2025 The Author(s). *Acta Physiologica* published by John Wiley & Sons Ltd on behalf of Scandinavian Physiological Society.

mainly unaltered in non-dialysis dependent CKD patients and CKD patients on peritoneal dialysis. The meta-analysis confirmed increased ROS generation in basal conditions predominantly in CKD patients on hemodialysis (Hedges $g = 1.20$, 95% CI: [0.32; 2.09]), with an insufficient study number for a clear comparison to CKD patients on peritoneal dialysis. However, upon neutrophil stimulation with sterile inflammatory triggers, ROS production was also increased in neutrophils from patients on peritoneal dialysis (Hedges $g = 0.89$, 95% CI: [0.34; 1.43]).

Conclusion: Increased degranulation and basal ROS formation were observed in neutrophils of CKD patients on hemodialysis, which could contribute to their increased cardiovascular risk. Future studies should compare neutrophil activity in patients of different CKD stages and comorbidities also in relation to cardiovascular outcomes.

1 | Introduction

Chronic kidney disease (CKD) is characterized by kidney damage or a reduced kidney filtration function for more than 3 months and has a substantial impact on health and survival outcomes [1]. It has a global prevalence of ~13.4% [2] and a high socio-economic and environmental burden. CKD is a progressive disease and is classified in CKD stages 1–5 based on the remaining glomerular filtration rate (GFR): patients can present with a normal to mild kidney dysfunction (CKD1-2), a mild–moderate or moderate–severe kidney dysfunction (CKD3a/b), a severe kidney impairment (CKD4) or complete kidney failure (CKD5) [3]. In this last stage, patients require kidney replacement therapy such as dialysis (hemodialysis (HD) or peritoneal dialysis (PD)) and kidney transplantation for survival. However, even with these interventions, life expectancy remains shortened, particularly due to an increase in cardiovascular risk that starts already in the early stages of CKD and grows exponentially with the progression of kidney dysfunction [4].

Overall, CKD patients show a chronic low-grade inflammation and innate immune activation [5], leading to progressive atherosclerosis [6, 7] and an increased risk of cardiovascular and thrombotic complications [4, 6, 8, 9]. Neutrophil granulocytes are the most abundant leukocytes in the blood and play an important role in cardiovascular disease (CVD) including atherosclerosis and thrombotic risk through the production of reactive oxygen species (ROS), the release of pro-inflammatory molecules (e.g., neutrophil elastase and myeloperoxidase) and the formation of neutrophil extracellular traps (NETs) [10, 11]. Increased neutrophil numbers [12, 13] and alterations in neutrophil activation responses have been reported [10] in CKD, though with a wide variety in readouts. Therefore, we executed a systematic review on the effects of CKD on neutrophil phenotype and function. We also performed a meta-analysis of neutrophil ROS production in CKD patients, since this was the most frequently studied readout of neutrophil responses in CKD and because of the critical role of ROS production in the context of CVD.

2 | Methods

This study was performed according to the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guidelines.

2.1 | Systematic Review: Search Strategy, Eligibility Criteria and Data Extraction

PubMed and the Web of Science were searched for studies describing the effects of CKD on human neutrophil function published 01/1990–09/2023, with Table S1 providing full details on the systematic search strategy as well as in- and exclusion criteria for both the systematic review and the meta-analysis. In short, studies were considered eligible when the phenotype or function of peripheral blood neutrophils was assessed in CKD patients in comparison to healthy volunteers, with readouts including—but not restricted to—surface activation marker expression, degranulation, ROS production, NET formation, apoptosis, and phagocytosis, and this both in basal as well as stimulated conditions (Figure 1). Studies were selected independently by two reviewers (J.S.L., P.M.) based on the predefined eligibility criteria (Table S1, Figure 1), with eventual disagreements resolved by consulting a third reviewer (H.N.). Additional relevant publications were identified by crosschecking the reference lists of the retrieved studies and relevant reviews (J.S.L., H.N.).

Data extracted from full-text articles include patient characteristics and study size, as well as findings on neutrophil apoptosis, activation markers, phagocytic activity, ROS production, degranulation, and NET release (Tables S2–S8, J.S.L., double-checked by C.V.C.J.).

2.2 | Meta-Analysis: Data Extraction and Quality Assessment

Since ROS production (i) was the most studied readout, (ii) contributes to CVD pathophysiology, and (iii) showed a high data variability in the systematic review, a meta-analysis with different subgroup analyses was performed for neutrophil ROS production in CKD. The meta-analysis could only be performed in CKD patients on dialysis compared to healthy controls, given the insufficient number of datasets for non-dialysis dependent CKD patients. Studies measured intracellular ROS (by flow cytometric analysis of 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA) or dihydrorhodamine oxidation), extracellular ROS (superoxide dismutase (SOD)-inhibitable cytochrome C reduction, lucigenin-enhanced chemiluminescence) or total ROS (luminol-enhanced chemiluminescence, dihydroethidium assays and DCFH-DA assays using plate readers, without addition of membrane-impermeable ROS scavengers) [14].

17481716, 2025, 6, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/apha.70057 by Jane Sophie Lauxen - Rwth Aachen University Zentraler Rechnungseingang , Wiley Online Library on [25/05/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

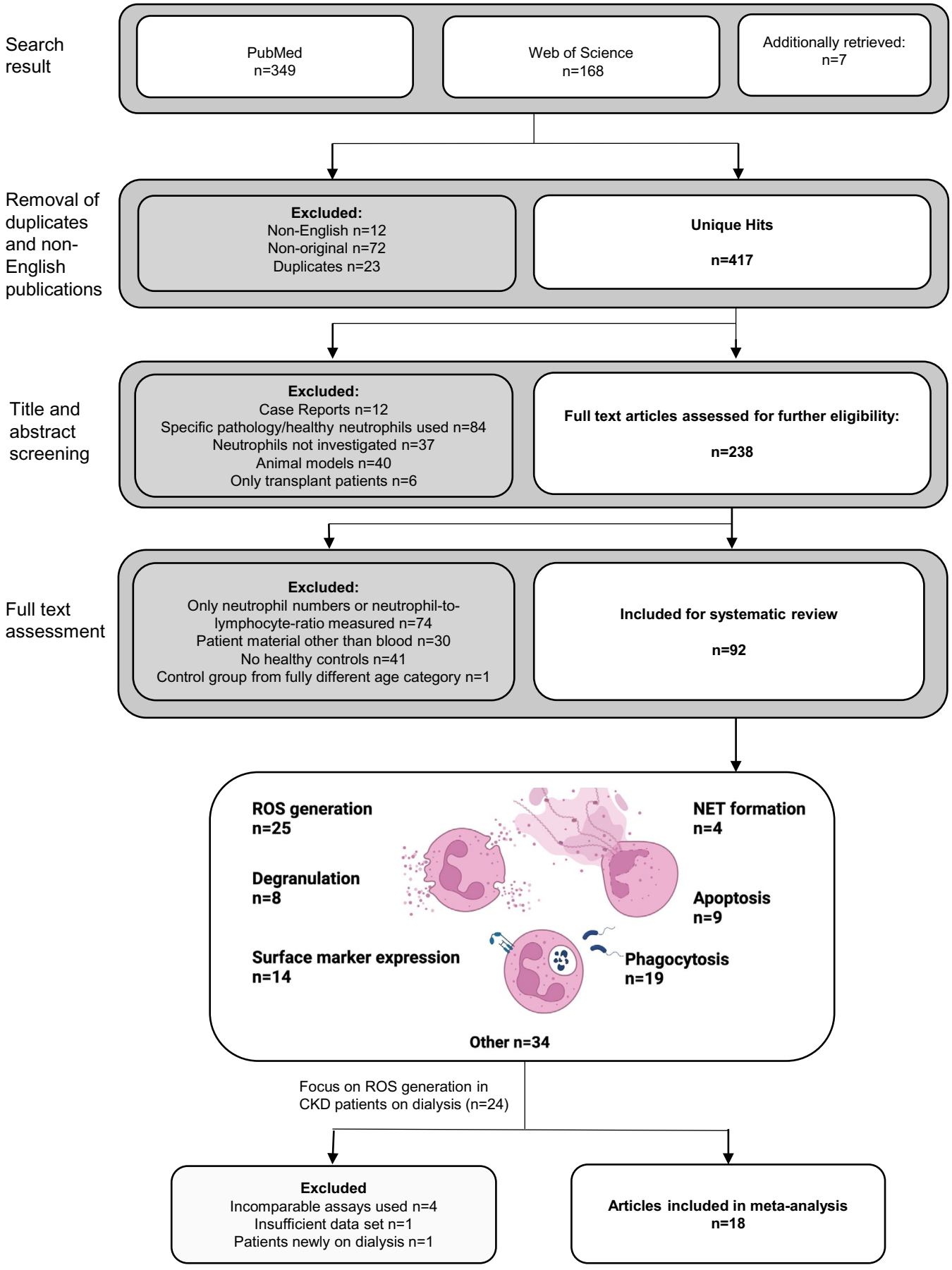


FIGURE 1 | Flow chart of study selection for systematic review and meta-analysis of neutrophil function in CKD. CKD, chronic kidney disease; NET, neutrophil extracellular traps; ROS, reactive oxygen species.

17481716, 2025, 6, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/apha.70057 by Jane Sophie Lauxen - Rwth Aachen University Zentraler Rechnungseingang , Wiley Online Library on [25/05/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

For the meta-analysis, study quality assessment included checking the completeness of the description of the patient population and the methods. Study size and values with mean and standard deviation (SD) or standard error of the mean (SEM) were extracted from the manuscript text, tables or graphical representations (J.S.L., double-checked by S.V./P.M.). SEM was converted to SD for inclusion in the meta-analysis. Furthermore, an N-number correction for multiple comparisons was applied where appropriate (i.e., when multiple CKD cohorts were compared to the same control group or when the same parameter (e.g., intracellular ROS) was measured in different ways).

2.3 | Meta-Analysis: Calculation and Statistical Analysis

Statistical analysis and graphical representation were performed in Rstudio v1.3.1093 using the 'meta', 'metafor' and 'dmetar' packages [15, 16]. The effect size of neutrophil ROS production in CKD patients vs. controls was calculated as standardized mean difference using Hedge's g with 95% confidence intervals. As we anticipated considerable between-study heterogeneity, the data were pooled using a random effects model (Hartung-Knapp-Sidik-Jonkman). I^2 was reported as a measure for heterogeneity across studies. As influencer outlier analysis, a Baujat plot and leave-one-out analysis were performed. Potential publication bias was examined in a funnel plot and Egger's test.

3 | Results

3.1 | Neutrophil Functional Changes in CKD: A Systematic Review

Using defined search and eligibility criteria (Table S1), we identified 524 articles examining neutrophil function in CKD. After exclusion of duplicates, non-English, and non-original publications, and after an initial screening based on title and abstract, the full text of 238 articles was assessed. Of these, 146 were excluded because they only measured neutrophil-to-lymphocyte ratio or neutrophil numbers ($n = 74$), used patient material other than blood ($n = 30$), had no healthy control group ($n = 41$) or used a non-adult control group vs. an adult patient group ($n = 1$). Thus, 92 studies were included in the systematic review (Figure 1). The most studied neutrophil response was ROS generation ($n = 25$), followed by phagocytosis ($n = 19$), surface marker expression ($n = 14$), apoptosis ($n = 9$), degranulation ($n = 8$) and NET formation ($n = 4$).

Figure 2 and Table 1 visually summarize the overall findings of the systematic review. Table S2 classifies the individual studies according to their effect (increase, decrease, unaltered) on each of the neutrophil readouts, whereas Tables S3–S8 provide full study details for each of the neutrophil readouts. A more detailed results description with additional details on the individual findings is provided in the Supplementary Results.

3.2 | Phagocytosis

Neutrophil phagocytic capacity was mostly found unaltered in non-dialysis CKD patients (unaltered: $n = 4$ studies [29, 34–36]; vs. reduced: $n = 2$ studies [27, 37]) and in CKD patients on PD (unaltered: $n = 5$ [27, 29, 34, 35, 37]). Instead, it was reduced in 67% (10 of 15) of studies in dialysis patients on HD (reduced: $n = 10$ [29, 30, 34–36, 38–42] vs. unaltered: $n = 5$ [25, 43–46]) (Figure 2, Tables 1, S2 and S3).

3.3 | Apoptosis

Neutrophil apoptosis was increased consistently in non-dialysis CKD patients [13, 29, 47–49], but only in a subset of studies in CKD patients on HD or PD (increased in HD [13, 49, 50] or increased in PD [13], vs. unaltered in HD [20, 26, 29, 48, 51] or PD [29, 48]; Figure 2, Tables 1, S2 and S4).

3.4 | Surface Activation Markers

CD11b, CD18, CD66b, and CD88 were the most frequently studied activation markers on neutrophils in CKD. Their analysis revealed generally increased neutrophil activation in non-dialysis CKD patients in both basal conditions (increased: $n = 4$ [49, 52–54] vs. reduced: $n = 1$ [55]) as well as in conditions of stimulation (increased: $n = 2$ [53, 54]). In contrast, neutrophils from HD patients showed in basal conditions only in half of

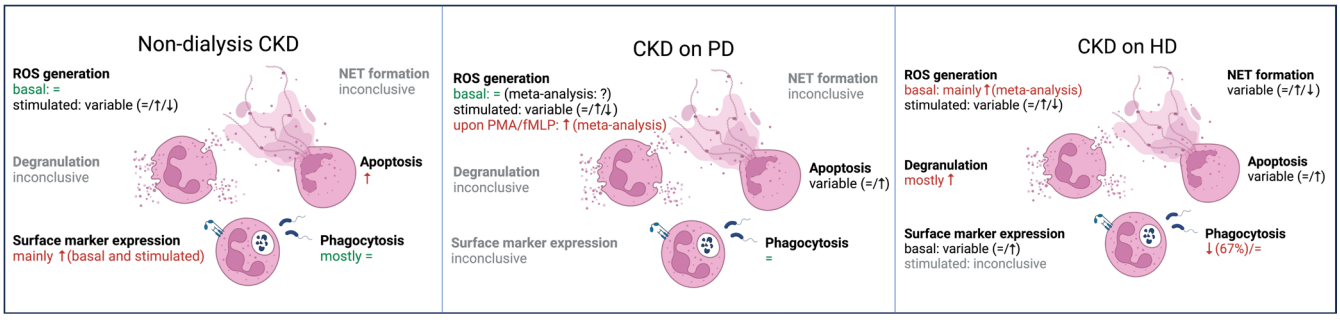


FIGURE 2 | Summary of studies reporting on neutrophil parameters in specific CKD patient cohorts (compared to healthy controls). Graphical summary of Table 2. "Inconclusive" indicates no overall conclusion due to insufficient study number. CKD, chronic kidney disease; HD, hemodialysis; NET, neutrophil extracellular traps; n.s., not specified; PD, peritoneal dialysis; ROS, reactive oxygen species.

17481716, 2025, 6, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/apha.70057 by Jane Sophie Lauxen - Rwth Aachen University Zentraler Rechnungseingang , Wiley Online Library on [25/05/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

TABLE 1 | Summary of studies reporting on neutrophil parameters in specific CKD patient cohorts (compared to healthy controls).

Neutrophil response	Non-dialysis CKD			Peritoneal dialysis		Hemodialysis			CKD: mixed[d] or n.s.	
Phagocytosis	4/6	2/6		5/5		5/15	10/15		1/2	1/2
Apoptosis (basal)	5/5			2/3	1/3	5/8	3/8		—	
Surface activation markers[a] (basal)	4/5	1/5		—		4/8	4/8		1/1	
Surface activation markers[a] (stimulated)	2/2 (LPS, fMLP)			—		1/1 (IL8)			1/1	
Degranulation (plasma markers)[b]	1/1			—		5/5			1/1	
Degranulation (neutrophil readouts)[b]	—			—		1/4	3/4		—	
NET formation (basal)	—			—		1/3	2/3		1/1	
NET formation (stimulated)	—			—		1/3	1/3	1/3	1/1	
ROS production (basal)	4/4			2/2		8/12	3/12	1/12	—	
ROS production (stimulation)[c]	3/7	2/7	3/7	3/5	2/5	1/5	7/17	2/17	9/17	—

Increased

Unaltered

Reduced

Note: The colors indicate whether the reported neutrophil response in specific CKD patient cohorts is increased, unaltered or reduced compared to healthy controls. (orange = increased, grey = unaltered, blue = reduced).
Abbreviations: CKD, chronic kidney disease; NET, neutrophil extracellular traps; n.s., not specified; ROS, reactive oxygen species.
[a]With inclusion of only CD11b, CD18, CD66b, and CD88 (Counted as increased if at least one of these was increased).
[b]Counted as increased if at least one of the markers was increased.
[c]Counted as increased (decreased) if at least with one of the stimuli, increased (decreased) ROS was observed in patients vs. controls. Thus, for a study with both an increased and decreased result depending on the stimulus, the study was counted in for both increased and decreased effects. Studies were counted as "unaltered" only if none of the tested conditions showed an increase or decrease in patients vs. controls.
[d]Mixed non-dialysis CKD, peritoneal dialysis, hemodialysis.

the studies an increase in (at least some of) these four activation markers (increased: $n = 4$ [18, 30, 33, 49]; unaltered: $n = 4$ [22, 52, 56, 57]). Insufficient data are available on stimulated neutrophils in HD patients ($n = 1$: unaltered [56]) as well as on neutrophils from PD patients (Figure 2, Tables 1, S2 and S5).

3.5 | Degranulation

Degranulation refers to the active release of granule contents in response to neutrophil stimulation. In addition to (i) an increased MPO or neutrophil elastase release upon neutrophil stimulation, we also included in this chapter the following readouts reported in the identified studies: (ii) reduced basal levels of MPO or neutrophil elastase in neutrophils, and (iii) increased levels of neutrophil elastase, MPO, lactoferrin, or histone-DNA in blood. All studies observed an increase in at least one of these readouts in either blood or isolated neutrophils in CKD patients compared to healthy controls ($n = 8$: 7× HD patients; 1× unspecified CKD stage) [24, 30, 58–63]. Only one study compared both non-dialysis CKD and HD patients to healthy controls, revealing increased plasma MPO levels only in HD patients [60] (Figure 2, Tables 1, S2 and S6).

3.6 | NET Formation

Overall, highly variable data were observed for NET formation by neutrophils from HD patients (basal NET formation: increased: $n = 2$ [22, 64] vs. unaltered: $n = 1$ [30]; stimulated NET formation: increased: $n = 1$ [64]; decreased: $n = 1$ [30]; unaltered: $n = 1$ [22, 65]; Figure 2, Tables 1, S2 and S7). No comparison was possible to PD patients or non-dialysis CKD patients due to a lack of data.

3.7 | ROS Production

In basal, unstimulated conditions, an increased ROS production was observed in 8 of 12 studies on HD patients, whereas no changes were detected in non-dialysis CKD patients ($n = 4$) or PD patients ($n = 2$). In conditions of neutrophil stimulation, findings on ROS production were highly variable in both non-dialysis as well as PD and HD patients (Figure 2, Tables 1, S2 and S8). Neutrophils produce ROS mainly via the NADPH oxidase 2 (NOX2) at the plasma membrane or in intracellular compartments, producing extracellular and/or intracellular ROS [14]. Furthermore, different stimuli have been used to analyze neutrophil ROS production. Since this may add

17481716, 2025, 6, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/apha.70057 by Jane Sophie Lauxen - Rwth Aachen University Zentraler Rechnungseingang , Wiley Online Library on [25/05/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

to the observed data variability, we subsequently performed a meta-analysis of neutrophil ROS production with subgroup analyses.

In summary, the systematic review identified primarily reduced phagocytosis capacity but increased neutrophil degranulation and basal ROS production in neutrophils from CKD patients on hemodialysis compared to controls (Figure 2). Instead, phagocytosis and basal ROS production were mainly unaltered in non-dialysis-dependent CKD patients and CKD patients on peritoneal dialysis (Figure 2).

3.8 | Neutrophil ROS Production in CKD: A Meta-Analysis

With ROS production being the most studied neutrophil readout and an important contributor to CVD pathophysiology, we performed a meta-analysis on ROS generation to summarize the available data and enable subgroup analyses to identify potential factors contributing to data heterogeneity in current literature. Since only eight studies investigated non-dialysis dependent CKD patients, the meta-analysis focused on ROS in dialysis patients ($n = 24$ studies, with patients either on HD or PD). A total of 18 studies used comparable methods for ROS measurements and provided sufficient details on datasets for inclusion in the meta-analysis (Figure 1, Table 2). The other studies had to be excluded based on incomparable assay use ($n = 4$; assays: oxygen consumption by Clark electrode, *C. albicans* as only stimulus, C5a and FLPEP as only stimulus, experimental setup unclear), insufficient data sets ($n = 2$: number of controls unclear, results only shown as median) and patients reported to be only on newly initiated dialysis ($n = 1$) (Figure 1, Table S1).

No publication bias was detected (Funnel plot in Figure S1; Eggers' test for Funnel plot asymmetry: $p = 0.089$). Influential outlier analyses revealed Cohen-Hagai et al. [19] as well as Kim et al. [22] as studies impacting most on the overall heterogeneity of the meta-analysis (Figure S2A). However, since these studies were not identified as unique strong influencers of the pooled result in both performed influential outlier tests (Figure S2A,B), no studies were excluded from the meta-analysis. Overall, the meta-analysis showed no significant difference in ROS production between neutrophils of dialysis patients and healthy controls, neither on overall level (Hedges $g = 0.06$; 95% CI: [−0.54; 0.66]) nor when analyzing specifically intra- or extracellular ROS (Figure S3, intracellular ROS: Hedges $g = -0.08$; 95% CI: [−0.95; 0.80]; extracellular ROS: Hedges $g = 0.07$; 95% CI: [−0.73; 0.88]).

However, the meta-analysis revealed an overall high data heterogeneity ($I^2 = 93\%$) and highlighted that even within the same study conflicting results could be detected dependent on the type of dialysis performed [29] (i.e., HD vs. PD), whether resting vs. stimulated neutrophils were examined [25] or dependent on which stimulus was applied [13, 20, 28, 31]. A second meta-analysis with subgrouping of studies based on the type of dialysis could not reveal an overall significant difference in neutrophil ROS generation in either HD or PD patients compared to healthy controls (HD: Hedges $g = -0.04$, 95% CI: [−0.81; 0.73]; PD: Hedges $g = 0.39$, 95% CI: [−0.09; 0.87], Figure 3), but again highlighted the presence and type of stimulus as an important influencing factor.

We therefore subdivided the included studies depending on the absence vs. presence as well as the type of stimulus. This revealed a significantly increased ROS production in resting neutrophils from dialysis patients (of which 91% HD patients) vs. healthy controls (Figure 4A; resting: Hedges $g = 1.20$, 95% CI: [0.32; 2.09]). Upon neutrophil stimulation, data remained highly variable (Figure S4). Stimuli included both direct bacterial or fungal triggers as well as stimuli that may rather reflect sterile inflammatory conditions, being PMA and fMLP (officially called fMLF, a prototypical formyl peptide used in research, with similar formylated peptides produced by mitochondria and released upon cell injury [66]). When focusing specifically on these latter, neutrophils from PD patients showed an overall increased ROS production compared to healthy controls (Figure 4B; PD: Hedges $g = 0.89$, 95% CI: [0.34; 1.43], $I^2 = 0\%$). In contrast, no significant effect could be observed in HD patients: ROS production upon neutrophil stimulation with PMA, fMLP, or fMLP following prior TNFα stimulation was identified to be significantly decreased in 7 out of 15 datasets on HD patients, though unaltered ($n = 5$ datasets) or increased ($n = 3$ datasets) in the remaining 8 datasets. (Figure 4B; HD: Hedges $g = -0.58$; 95% CI: [−1.61; 0.46], $I^2 = 94\%$).

All combined, these meta-analyses indicate that in basal, unstimulated conditions, ROS production is increased in neutrophils from CKD patients on HD, while CKD patients on PD are insufficiently investigated. Upon sterile inflammatory stimulation of neutrophils, ROS production is increased in CKD patients receiving PD, with findings yet inconclusive for CKD patients on HD due to high data variability (Figure 2).

4 | Discussion

Our systematic review revealed that neutrophils from CKD patients on HD generally show reduced phagocytosis capacity (reflecting a reduced response to an acute infection) but increased neutrophil degranulation and basal ROS production (reflecting an increased basal inflammatory response) compared to healthy controls. In contrast, neutrophils from non-dialysis-dependent CKD patients and CKD patients on PD mostly showed unaltered phagocytosis and basal ROS production (Figure 2). A meta-analysis confirmed an increased basal ROS production in neutrophils of CKD patients on HD. Upon neutrophil stimulation with sterile inflammatory triggers, ROS production was also increased in neutrophils from PD patients.

Increased basal ROS production in resting neutrophils from HD patients points to enhanced basal cellular priming, and the procedure of HD can indeed be expected to affect neutrophil phenotype more than in the case of PD. Contributing factors include cellular stress induced by the HD procedure during which neutrophils are regularly exposed to shear stress and exogenous materials (dialysis membrane, (heparinized) plastic tubing, etc.) for a longer time. This is absent in non-dialysis CKD patients, but also in PD patients in whom the peritoneum functions as an endogenous dialysis membrane. Other causes of HD-induced

17481716, 2025, 6, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/apha.70057 by Jane Sophie Lauxen - Rwth Aachen University Zentraler Rechnungseingang , Wiley Online Library on [25/05/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

TABLE 2 | Characteristics of studies analyzing neutrophil ROS production in dialysis patients, as included in the meta-analysis.

Study	Patient cohort	Neutrophils in full blood/ isolated neutrophils	ROS Assay (I, intracellular) (E, extracellular) (T, total)	Stimulus	Overall study conclusion on ROS response in patients compared to healthy controls (Basal/Stimulated)	Dialysis procedure
Cohen et al. [17]	HD	Full blood analysis	DHR oxidation (FC) (I)	*E. coli* & PMA	Decreased (stimulated)	High-flux membranes (nitrocellulose triacetate)
Cohen-Mazor et al. [18]	HD	PMNL	SOD-inhibitable cytochrome c reduction (E)	PMA	Increased (stimulated)	Low-flux polysulfone membranes
Cohen-Hagai et al. [19]	HD	Full blood analysis	DHR oxidation (FC) (I)	*E. coli* & PMA	Decreased (stimulated)	n.s.
Guo et al. [20]	HD	PMNL	DCFH-DA oxidation (FC) (I)	PMA, fMLP & *S. aureus*	Increased (unstimulated + stimulated with *S. aureus* and fMLP), unaltered (stimulated with PMA)	n.s.
Haynes et al. [21]	PD	PMNL	Lucigenin-enhanced chemiluminescence (E)	fMLP	Increased (stimulated)	—
Kim et al. [22]	HD	PMNL	DCFH-DA oxidation (plate reader) (T)	Unstimulated	Increased (unstimulated)	Standard bicarbonate dialysis & semisynthetic membranes
Kristal et al. [23]	HD	PMNL	SOD-inhibitable cytochrome c reduction (E)	PMA	Increased (stimulated)	Polysulfone or cellulose triacetate dialysis membranes
Otaki et al. [24]	HD	PMNL	DCFH-DA oxidation (FC) (I)	Unstimulated	Unaltered (unstimulated)	Either cellulose triacetate, excebrane, or polymethylmethacrylate membranes and bicarbonate dialysate
Patruta et al. [25]	HD	PMNL	SOD-inhibitable cytochrome c reduction (E)	PMA	Increased (unstimulated) Decreased (stimulated)	Bicarbonate hemodialysis using biocompatible membrane material made of polysulfone or cellulose triacetate
Perianayagam et al. [26]	HD	Full blood analysis	DCFH-DA oxidation (FC) (I)	Unstimulated	Increased (unstimulated)	High-flux dialyzers

(Continues)

17481716, 2025, 6, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/apha.70057 by Jane Sophie Lauxen - Rwth Aachen University Zentraler Rechnungseingang , Wiley Online Library on [25/05/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

TABLE 2 | (Continued)

Study	Patient cohort	Neutrophils in full blood/ isolated neutrophils	ROS Assay (I, intracellular) (E, extracellular) (T, total)	Stimulus	Overall study conclusion on ROS response in patients compared to healthy controls (Basal/Stimulated)	Dialysis procedure
Porter et al. [27]	PD	PMNL	Luminol-enhanced chemiluminescence (T); DCFH-DA oxidation (FC) (I)	*S. epidermidis*	Unaltered (stimulated)	—
Rao et al. [28]	HD	PMNL	SOD-inhibitable cytochrome c reduction (E)	PMA, fMLP	Decreased (stimulated PMA), unaltered (stimulated fMLP)	Polysulphone (PS), cuprophane (CU), cellulose (acetate, triacetate) (CA/CT)
Sardenberg et al. [29]	HD PD	Full blood analysis	DCFH-DA oxidation (FC) (I)	Unstimulated, *S. aureus*, PMA, fMLP	Increased (stimulated with bacterial and fMLP+basal for CAPD), Unaltered (stimulated + unstimulated HD, stimulated with PMA for CAPD)	HD: polysulfone dialyzers CAPD: exchanges of glucose, lactate-based solutions that usually included one 4.25% glucose solution exchange
Sela et al. [13]	PD HD	PMNL	SOD-inhibitable cytochrome c reduction (E)	unstimulated, PMA, Zymosan	Increased (stimulated PMA), Decreased (stimulated Zymosan)	CAPD: 8 L/d in four exchanges (three isotonic 1.36% and one hypertonic 3.86% glucose solutions); HD: low-flux polysulfone membranes with bicarbonate dialysate
Talal et al. [30]	HD	Neutrophils	Extracellular ROS with chemilumescence (E)	PMA, LPS	Decreased (stimulated)	n.s.
Ward et al. [31]	HD	PMNL	Cytochrome c reduction (E)	TNF, fMLP	Unaltered (unstimulated), Decreased (stimulated)	Reused dialyzers containing cellulose acetate membrane
Ward et al. [32]	HD	Full blood analysis	DCFH-DA oxidation (FC) (I)	Unstimulated, *S. aureus*	Increased (unstimulated + stimulated)	High-flux dialyzers containing polysulfone and cellulose triacetate membranes
Yoon et al. [33]	HD	Full blood analysis	DHE oxidation, DHR oxidation (FC) (I)	Unstimulated	Increased (unstimulated)	Cellulose acetate dialyzers

Note: For ROS location: (I) intracellular; (E) extracellular; (T) total = intra- and extracellular. All studies were observational studies.
Abbreviations: DCFH-DA, 2′,7′-dichlorodihydrofluorescein diacetate; DHE, dihydroethidium; DHR, dihydrorhodamine; EDTA, ethylenediaminetetraacetic acid; FC, flow cytometry; HD, hemodialysis; MFI, mean fluorescence intensity; n.s., not specified; obs., observational; PD, peritoneal dialysis; PMNL, polymorphonuclear leukocyte.

17481716, 2025, 6, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/apha.70057 by Jane Sophie Lauxen - Rwth Aachen University Zentraler Rechnungsempfang , Wiley Online Library on [25/05/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

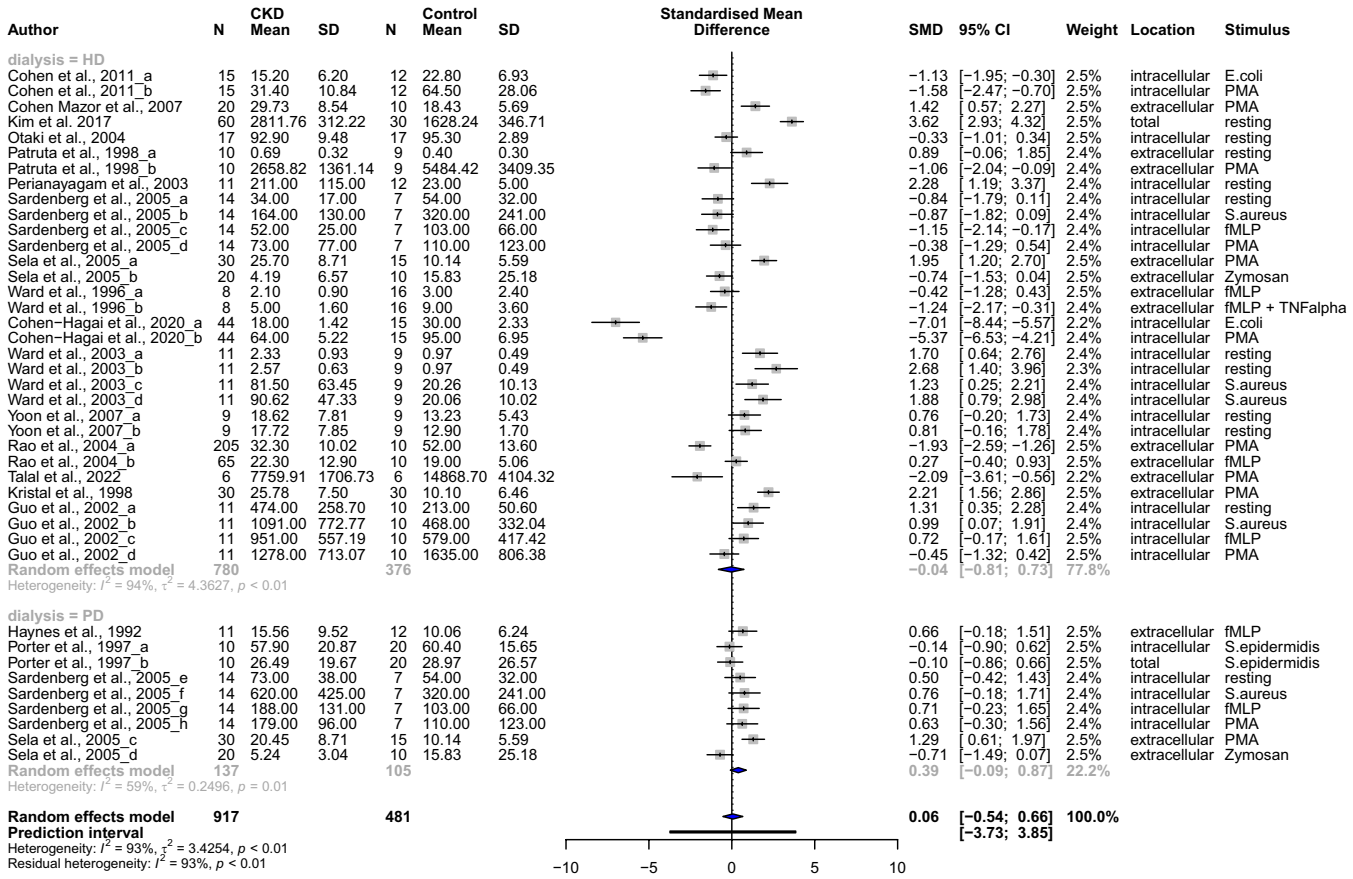


FIGURE 3 | Meta-analysis shows no overall significant difference in neutrophil ROS production between CKD patients on either hemodialysis or peritoneal dialysis versus controls. Forest plot of neutrophil ROS production in dialysis patients compared to healthy controls, with subgroup analysis of the effect of dialysis type. CI, confidence interval; CKD, chronic kidney disease; *E. coli*, *Escherichia coli*; HD, hemodialysis; fMLP, N-formyl-methionyl-leucyl-phenylalanine; PD, peritoneal dialysis; PMA, Phorbol 12-myristate 13-acetate; *S. aureus*, *Staphylococcus aureus*; SD, standard deviation; *S. epidermidis*, *Streptococcus epidermidis*; TNFα, tumor necrosis factor α.

cellular stress could be uremic retention solute accumulation in between HD procedures (with higher fluctuation in circulating levels compared to the continuous PD procedure), complement activation, chronic low-grade inflammation [67, 68] or iron overload [20, 24], amongst others. The high variability in neutrophil readouts (including also apoptosis, surface activation markers, NET formation and phagocytosis) observed in HD patients may be due to differences in dialysis membranes [40] and modalities used, which may trigger alterations in uremic retention solute removal or dialysis-induced inflammatory reactions and cellular stress. In line, a transient increase in neutrophil activation markers during the HD procedure has been observed dependent on the dialysis membrane used [52].

Neutrophil priming and increased basal ROS levels could also explain a secondary functional impairment with a reduced fold-change upregulation of ROS production upon neutrophil stimulation (which has been described for NET formation in HD patient-derived neutrophils [22]) or even reduced ROS production by stimulated neutrophils in HD patients [31] (Table 1, Figure 4B). This supports the hypothesis that neutrophils of HD patients may already be in a primed state due to the uremic and/or inflammatory milieu they are consistently exposed to, an effect that could be mimicked when incubating neutrophils from healthy individuals in plasma from HD patients [31].

On the other hand, basal cellular priming can also trigger increased secondary responses ("positive priming"), with multiple studies on neutrophils in HD patients also reporting on increased stimulation-induced ROS production (Table 1, Figure 4B). However, in these studies, the results were dependent on the stimulus used (Figure 4A, Table S8) [13, 20, 28, 31]. For example, Sela et al. found an increase in ROS production upon stimulation of HD patient-derived neutrophils with PMA (mimicking sterile inflammation) but a decrease with Zymosan (a component of the cell wall of yeast, mimicking microbial infection) [13]. The latter may be linked to the high susceptibility of CKD patients to acute infection [69, 70].

The overall high variability in findings on neutrophil activity readouts in CKD between different studies could be due to a high patient population heterogeneity with regard to CKD stage, primary underlying CKD pathology, existing comorbidities such as hypertension, obesity, diabetes, and CVD [8], as well as medication. For example, diabetes—a known cause and promoter of inflammation and oxidative stress [71], neutrophil activation [72] as well as of CKD [8]—was an exclusion criterium in only 17% of the included studies and could well be a variable factor between different studies of CKD patients. Towards the use of immunosuppressants, six of the 18 studies included in the meta-analysis explicitly excluded patients on immunosuppressive therapy, and

17481716, 2025, 6, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/apha.70057 by Jane Sophie Lauxen - Rwth Aachen University Zentraler Rechnungseingang , Wiley Online Library on [25/05/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

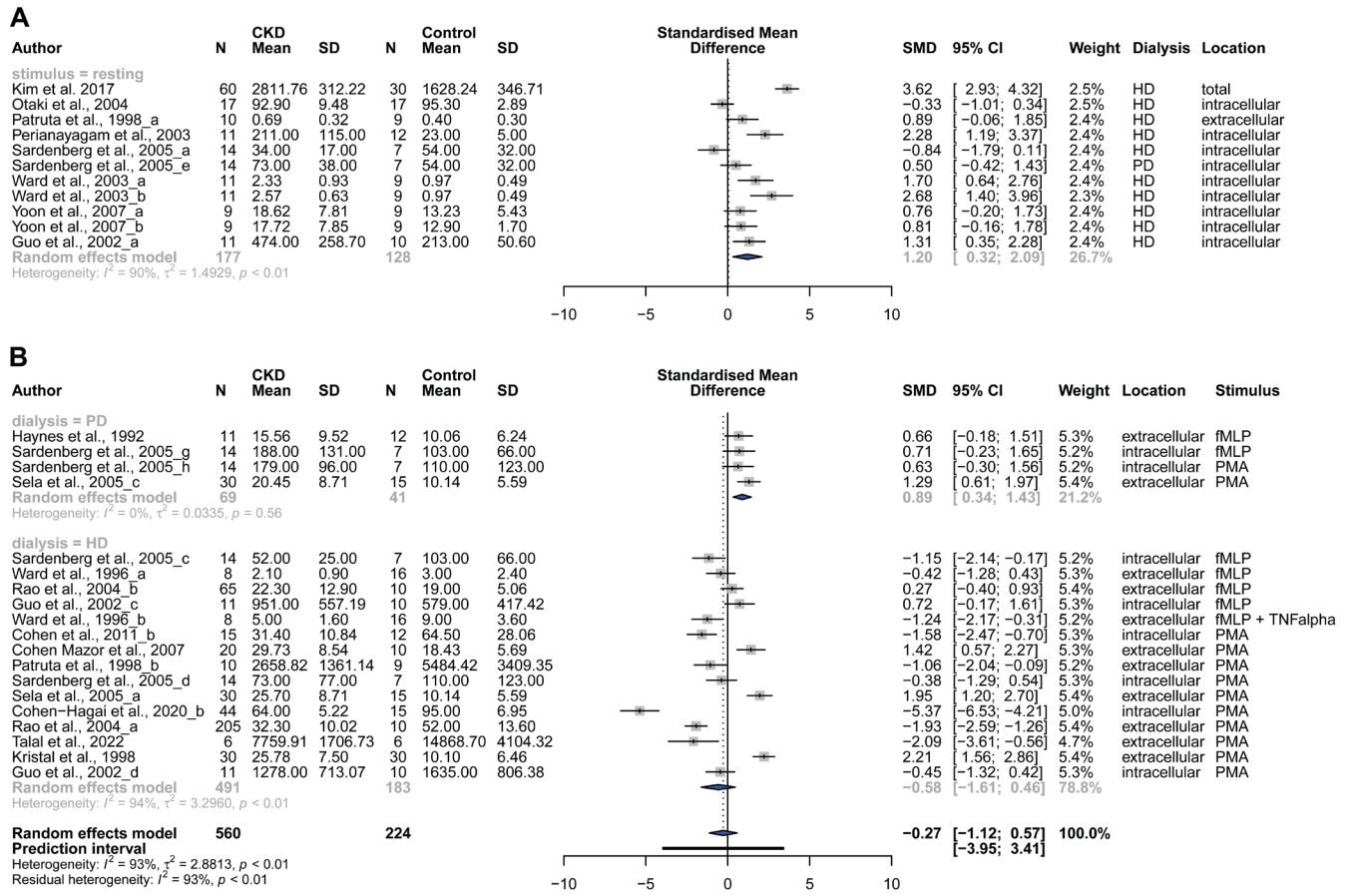


FIGURE 4 | Meta-analysis of ROS production by resting or stimulated neutrophils from CKD patients on dialysis. (A) Meta-analysis shows increased ROS production by resting neutrophils from predominantly hemodialysis-patients versus controls. Forest plot of neutrophil ROS production in dialysis patients compared to healthy controls, with analysis of the subgroup "resting neutrophils". Provided is a zoomed extract from the full meta-analysis of the effect of stimulus presence and type on ROS generation in neutrophils, as displayed in Figure S4. (B) Meta-analysis shows increased ROS generation in neutrophils of PD patients, when stimulated with stimuli reflecting sterile inflammatory conditions. Forest plot of neutrophil ROS production in dialysis patients compared to healthy controls upon neutrophil stimulation with fMLP, fMLP and TNFα or PMA. CI, confidence interval; CKD, chronic kidney disease; fMLP, N-formyl-methionyl-leucyl-phenylalanine; HD, hemodialysis; PD, peritoneal dialysis; PMA, Phorbol 12-myristate 13-acetate; SD, standard deviation, TNFα, tumor necrosis factor α.

another two excluded patients with immunological disorders or with diseases associated with neutrophil dysfunction. Moreover, the dialysis type, duration on dialysis [73], and for HD patients also the timing of neutrophil analysis in relation to dialysis application and the applied dialysis membrane (which varied throughout the studies included in the meta-analysis) may contribute to the high interstudy data heterogeneity in the meta-analysis.

Also, the methods for analyzing ROS levels in neutrophils varied throughout the included papers, both in terms of analyzing extracellular vs. intracellular ROS (or a combination of both), as well as in terms of the specific assay used and ROS type measured. Neutrophils mainly produce ROS via the NADPH oxidase (NOX2), which reduces oxygen to the superoxide radical ($O_2^{\bullet-}$). Superoxide can either be produced at the plasma membrane (extracellular ROS) or in intracellular compartments (intracellular ROS) [14]. Superoxide rapidly dismutates to hydrogen peroxide (H_2O_2) by superoxide dismutase (SOD) or spontaneously, and can also convert to other types of ROS (e.g., peroxynitrite ($ONOO^-$)). PMA is a potent inducer of both intra- and extracellular ROS production. The produced ROS is subsequently important for neutrophil function. For example, NADPH-oxidase-dependent intracellular ROS production is important for the killing capacity of neutrophils towards microbes, as well as for PMA-induced NETosis [14]. In the meta-analysis, 9 of 18 studies are based on the detection of superoxide. With superoxide rapidly converting to peroxynitrite or H_2O_2, measuring superoxide is a more unreliable ROS detection method compared to H_2O_2, which is less reactive. Overall, the use of chemical probes to measure ROS has several pitfalls, as extensively discussed before [74, 75]. Briefly, lack of specificity, no subcellular localization, indirect mechanism of oxidation, photo-oxidation, and the fact that these probes are prone to artifacts are amongst the main problems (Table S9). For example, probes such as dihydrorhodamine also react with reactive nitrogen species. It is also important to mention that ROS production and removal are highly compartmentalized reactions and are unlikely to have effects far from the site where they are generated [76]. Understanding these limitations helps to interpret the great variability in some of the conditions discussed above. Of note, a lack of ROS changes in some conditions does not necessarily mean that

17481716, 2025, 6, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/apha.70057 by Jane Sophie Lauxen - Rwth Aachen University Zentraler Rechnungseingang , Wiley Online Library on [25/05/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

ROS do not play an important role in CKD. It would be interesting to analyze specific ROS at defined cellular compartments to understand the causal role of different redox systems in CKD (e.g., mitochondria versus NADPH oxidase). Finally, ROS are important signaling molecules; therefore, understanding what the "optimal" ROS levels for cellular function are and when this threshold is shifted towards pathophysiology is still a highly active field of research [77].

Circulating blood neutrophils exhibit a remarkable heterogeneity. At any given time, various phenotypes of neutrophils coexist, with young vs. aged neutrophils representing a classic example of phenotypic heterogeneity at steady state. In disease states, such heterogeneity becomes more complex as discrete phenotypes of activated, primed, and immature neutrophils appear [78, 79]. Yet, these states are not just phenotypes discernable by surface markers, but indeed exhibit differential responses in terms of NET release, phagocytosis, adhesion, and ROS production. Thus, analyses based on assessing neutrophil functionality on a bulk scale may not suffice to identify altered responsiveness of neutrophil phenotypes. As an example, ROS production is often assessed in plate reader assays where a signal is recorded over time and possibly normalized by the number of cells seeded. Such population-based analysis disregards differential responses of subpopulations, information only single cell-based assays can reveal. Thus, flow cytometry-based analysis of ROS production in different activation and maturation stages of neutrophils would yield more robust and detailed information of neutrophil responses. In addition to subset-specific responses, methods of neutrophil extraction have tremendous effects on neutrophil responses. Lysis of red blood cells, density gradient centrifugation, and temperature changes all impact the phenotypic appearance of neutrophils and functionality, and different isolation protocols across studies assessed here may at least in part explain data heterogeneity. Moreover, the isolation protocol may even get rid of certain neutrophil subsets. As an example, low-density granulocytes are found in the PBMC fraction rather than in the neutrophil fraction in density gradient-based isolation procedures. Finally, and in addition to what has been described above for ROS production, it is key to provide details on experimental procedures. Phagocytosis assays, for example, are often performed with bacteria-based bioparticles. As a considerable number of these bioparticles are only bound to neutrophil surfaces and not internalized, newer versions of these bioparticles are conjugated with a pH-sensitive dye allowing for the recording of the acidification in the phagolysosome. Yet, in the body, bacteria are always opsonized either with antibodies or with activated complement, reagents often not used in ex vivo phagocytosis assays. Thus, the use of different opsonins and consequently different phagocytosis pathways may explain part of the data heterogeneity observed here.

A limitation of our study is the large time span between the included studies, ranging from 1990 until 2023. This may have impacted disease management (and thus characteristics of patients and their neutrophils), dialysis methods as well as research methods (e.g., differences in neutrophil isolation, different method of ROS analysis). Furthermore, non-dialysis dependent CKD patients were not further specified according to CKD stage. Finally, we classified the presence of different neutrophil granule markers and histone-DNA in blood as parameters for "degranulation". However, these are not necessarily related to degranulation or even secretion but could also result from neutrophil death or from other cell types (as e.g., MPO from monocytes).

5 | Conclusions

In conclusion, our study identified reduced phagocytic capacity but increased degranulation and basal ROS production in neutrophils from CKD patients on HD. This reflects a chronic inflammatory response but reduced responsiveness of neutrophils to an acute infection. Instead, increased basal ROS and reduced phagocytosis were mostly not detected in CKD patients on PD or non-dialysis-dependent CKD patients. Nonetheless, caution remains required since in general only a limited number of studies have addressed these patient groups, and additional studies on surface markers, NET formation, and degranulation markers are needed. A more detailed and side-by-side comparison of patients in stages CKD2–4 for different readouts of neutrophil function as well as studying neutrophil function in patients with CKD in relation to comorbidities (as diabetes) may provide further insight into the inherent effect of CKD on neutrophil activity.

We suggest future studies assessing the detailed neutrophil phenotype to combine the analysis of different activation markers on the neutrophil surface in whole blood, a fluorescent-based phagocytosis bead assay as well as an assay for NET formation on isolated neutrophils. Analysis is recommended in both basal conditions as well as upon activation with different stimuli, such as PMA vs. IL8 or fMLP, since different signaling mechanisms may be differentially affected by the underlying pathology. Although PMA is a frequently applied stimulus, it is a synthetic stimulus that directly activates protein kinase C without the aid of any membrane receptor. When analyzing ROS, a flow cytometry-based assay has the advantage to focus on neutrophils in a whole blood setting and to analyze the readout per cell. Analyzing mitochondrial versus NADPH-oxidase would be interesting, and given the inherent difficulties with ROS assays described above, a combination of different assays with appropriate controls is advisable. Depending on the research question, exclusion of patients on immunosuppressive therapy is recommended, and patient characteristics and clinical details (including age, sex, CKD stage, comorbidities and medication, time on dialysis and time of blood sampling relative to the dialysis sessions) should be reported.

To our knowledge, no specific outcome studies investigating the association of markers of increased neutrophil activity to cardiovascular outcome have been performed in CKD patients so far, although this would be of high clinical relevance. Patients with CKD have an increased cardiovascular risk, to which increased neutrophil activation, ROS production, and NET formation [10] but also altered neutrophil interactions with endothelium [80] and platelets may contribute. Thus, a further clarification of the impact of CKD on these neutrophil responses also in CKD2-4 could support future management of chronic inflammation and cardiovascular risk in CKD patients.

17481716, 2025, 6, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/apha.70057 by Jane Sophie Lauxen - Rwth Aachen University Zentraler Rechnungseingang , Wiley Online Library on [25/05/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Author Contributions

Jane Sophie Lauxen: investigation, data curation, formal analysis, visualization, writing – original draft, writing – review and editing. **Sonja Vondenhoff:** validation, writing – review and editing. **Carolina Victoria Cruz Junho:** investigation, validation, writing – review and editing. **Philipp Martin:** validation, writing – review and editing. **Susanne Fleig:** writing – review and editing. **Katharina Schütt:** writing – review and editing. **Ulrike Schulze-Späte:** writing – review and editing. **Oliver Soehnlein:** writing – review and editing. **Leticia Prates-Roma:** writing – review and editing. **Yvonne Döring:** writing – review and editing. **Constance C. F. M. J. Baaten:** methodology, software, visualization, writing – review and editing. **Heidi Noels:** conceptualization, funding acquisition, methodology, supervision, validation, writing – review and editing.

Acknowledgments

We thank Prof. Dr. Raymond Vanholder for a critical evaluation and constructive feedback to this manuscript. Open Access funding enabled and organized by Projekt DEAL.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data extracted from the included studies and used for all analyses are presented in detail in Table 2 and the meta-analysis figures. The extracted data values used in the meta-analysis are also publicly available as Supplemental Data Set via the University library of the RWTH Aachen (DOI: https://doi.org/10.18154/RWTH-2025-03170).

References

1. A. Francis, M. N. Harhay, A. C. M. Ong, et al., "Chronic Kidney Disease and the Global Public Health Agenda: An International Consensus," *Nature Reviews. Nephrology* 20 (2024): 473–485, https://doi.org/10.1038/s41581-024-00820-6.

2. N. R. Hill, S. T. Fatoba, J. L. Oke, et al., "Global Prevalence of Chronic Kidney Disease—A Systematic Review and Meta-Analysis," *PLoS One* 11, no. 7 (2016): e0158765, https://doi.org/10.1371/journal.pone.0158765.

3. L. A. Inker, B. C. Astor, C. H. Fox, et al., "KDOQI US Commentary on the 2012 KDIGO Clinical Practice Guideline for the Evaluation and Management of CKD," *American Journal of Kidney Diseases* 63 (2014): 713–735, https://doi.org/10.1053/j.ajkd.2014.01.416.

4. K. Matsushita, S. H. Ballew, A. Y. Wang, R. Kalyesubula, E. Schaeffner, and R. Agarwal, "Epidemiology and Risk of Cardiovascular Disease in Populations With Chronic Kidney Disease," *Nature Reviews. Nephrology* 18 (2022): 696–707, https://doi.org/10.1038/s41581-022-00616-6.

5. C. Zoccali and F. Mallamaci, "Innate Immunity System in Patients With Cardiovascular and Kidney Disease," *Circulation Research* 132 (2023): 915–932, https://doi.org/10.1161/circresaha.122.321749.

6. S. Thompson, M. James, N. Wiebe, et al., "Cause of Death in Patients With Reduced Kidney Function," *Journal of the American Society of Nephrology* 26 (2015): 2504–2511, https://doi.org/10.1681/asn.2014070714.

7. J. M. Valdivielso, D. Rodriguez-Puyol, J. Pascual, et al., "Atherosclerosis in Chronic Kidney Disease: More, Less, or Just Different?," *Arteriosclerosis, Thrombosis, and Vascular Biology* 39 (2019): 1938–1966, https://doi.org/10.1161/ATVBAHA.119.312705.

8. J. Jankowski, J. Floege, D. Fliser, M. Bohm, and N. Marx, "Cardiovascular Disease in Chronic Kidney Disease: Pathophysiological Insights and Therapeutic Options," *Circulation* 143 (2021): 1157–1172, https://doi.org/10.1161/CIRCULATIONAHA.120.050686.

9. H. Noels and J. Jankowski, "Increased Risk of Cardiovascular Complications in Chronic Kidney Disease: Introduction to a Compendium," *Circulation Research* 132 (2023): 899–901, https://doi.org/10.1161/circresaha.123.322806.

10. M. Thakur, C. V. C. Junho, S. M. Bernhard, M. Schindewolf, H. Noels, and Y. Döring, "NETs-Induced Thrombosis Impacts on Cardiovascular and Chronic Kidney Disease," *Circulation Research* 132 (2023): 933–949, https://doi.org/10.1161/circresaha.123.321750.

11. C. Silvestre-Roig, Q. Braster, A. Ortega-Gomez, and O. Soehnlein, "Neutrophils as Regulators of Cardiovascular Inflammation," *Nature Reviews. Cardiology* 17 (2020): 327–340, https://doi.org/10.1038/s41569-019-0326-7.

12. C. H. Yen, I. W. Wu, C. C. Lee, et al., "The Prognostic Value of Peripheral Total and Differential Leukocyte Count in Renal Progression: A Community-Based Study," *PLoS One* 16 (2021): e0258210, https://doi.org/10.1371/journal.pone.0258210.

13. S. Sela, R. Shurtz-Swirski, M. Cohen-Mazor, et al., "Primed Peripheral Polymorphonuclear Leukocyte: A Culprit Underlying Chronic Low-Grade Inflammation and Systemic Oxidative Stress in Chronic Kidney Disease," *Journal of the American Society of Nephrology* 16 (2005): 2431–2438, https://doi.org/10.1681/asn.2004110929.

14. C. Dahlgren, A. Karlsson, and J. Bylund, "Intracellular Neutrophil Oxidants: From Laboratory Curiosity to Clinical Reality," *Journal of Immunology* 202 (2019): 3127–3134, https://doi.org/10.4049/jimmunol.1900235.

15. Team R, "RStudio: Integrated Development for R," 2016, accessed October 6, https://www.rstudio.com.

16. M. Harrer, P. Cuijpers, T. Furukawa, and D. D. Ebert, "dmetar: Companion R package for the guide 'Doing Meta-Analysis in R'," 2019, accessed January 21, https://dmetar.protectlab.org/.

17. G. Cohen, J. Raupachova, D. Ilic, J. Werzowa, and W. H. Hörl, "Effect of Leptin on Polymorphonuclear Leucocyte Functions in Healthy Subjects and Haemodialysis Patients," *Nephrology, Dialysis, Transplantation* 26 (2011): 2271–2281, https://doi.org/10.1093/ndt/gfq731.

18. M. Cohen-Mazor, S. Sela, R. Mazor, et al., "Are Primed Polymorphonuclear Leukocytes Contributors to the High Heparanase Levels in Hemodialysis Patients?," *American Journal of Physiology. Heart and Circulatory Physiology* 294 (2008): H651–H658, https://doi.org/10.1152/ajpheart.00952.2007.

19. K. Cohen-Hagai, N. Nacasch, A. Sternschuss, et al., "Malnutrition and Inflammation in Hemodialysis Patients: Comparative Evaluation of Neutrophil Reactive Oxygen Formation," *Nutrition* 78 (2020): 110793, https://doi.org/10.1016/j.nut.2020.110793.

20. D. Guo, B. L. Jaber, S. Lee, et al., "Impact of Iron Dextran on Polymorphonuclear Cell Function Among Hemodialysis Patients," *Clinical Nephrology* 58 (2002): 134–142, https://doi.org/10.5414/cnp58134.

21. A. P. Haynes, I. Daniels, C. Porter, J. Fletcher, and A. G. Morgan, "Abnormal Cytoplasmic pH Regulation During Activation in Uremic Neutrophils," *Kidney International* 42 (1992): 690–699, https://doi.org/10.1038/ki.1992.336.

22. J. K. Kim, C. W. Hong, M. J. Park, Y. R. Song, H. J. Kim, and S. G. Kim, "Increased Neutrophil Extracellular Trap Formation in Uremia Is Associated With Chronic Inflammation and Prevalent Coronary Artery Disease," *Journal of Immunological Research* 2017 (2017): 8415179, https://doi.org/10.1155/2017/8415179.

23. B. Kristal, R. Shurtz-Swirski, S. M. Shasha, et al., "Interaction Between Erythropoietin and Peripheral Polymorphonuclear Leukocytes in Hemodialysis Patients," *Nephron* 81 (1999): 406–413, https://doi.org/10.1159/000045324.

17481716, 2025, 6, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/apha.70057 by Jane Sophie Lauxen - Rwth Aachen University Zentraler Rechnungseingang , Wiley Online Library on [25/05/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

24. Y. Otaki, T. Nakanishi, Y. Hasuike, et al., "Defective Regulation of Iron Transporters Leading to Iron Excess in the Polymorphonuclear Leukocytes of Patients on Maintenance Hemodialysis," *American Journal of Kidney Diseases* 43 (2004): 1030–1039, https://doi.org/10.1053/j.ajkd.2004.02.016.

25. S. I. Patruta, R. Edlinger, G. Sunder-Plassmann, and W. H. Hörl, "Neutrophil Impairment Associated With Iron Therapy in Hemodialysis Patients With Functional Iron Deficiency," *Journal of the American Society of Nephrology* 9 (1998): 655–663, https://doi.org/10.1681/asn.V94655.

26. M. C. Perianayagam, V. S. Balakrishnan, D. Guo, B. J. Pereira, and B. L. Jaber, "Quantification of Bax and Bcl2 in Polymorphonuclear Leukocytes From Haemodialysis Patients: Relation to Hydrogen Peroxide," *European Journal of Clinical Investigation* 33 (2003): 905–911, https://doi.org/10.1046/j.1365-2362.2003.01225.x.

27. C. J. Porter, R. P. Burden, A. G. Morgan, I. Daniels, and J. Fletcher, "Impaired Bacterial Killing and Hydrogen Peroxide Production by Polymorphonuclear Neutrophils in End-Stage Renal Failure," *Nephron* 77 (1997): 479–481, https://doi.org/10.1159/000190328.

28. M. Rao, D. Guo, B. L. Jaber, et al., "Dialyzer Membrane Type and Reuse Practice Influence Polymorphonuclear Leukocyte Function in Hemodialysis Patients," *Kidney International* 65 (2004): 682–691, https://doi.org/10.1111/j.1523-1755.2004.00429.x.

29. C. Sardenberg, P. Suassuna, M. C. Andreoli, et al., "Effects of Uraemia and Dialysis Modality on Polymorphonuclear Cell Apoptosis and Function," *Nephrology, Dialysis, Transplantation* 21 (2006): 160–165, https://doi.org/10.1093/ndt/gfi095.

30. S. Talal, K. Mona, A. Karem, et al., "Neutrophil Degranulation and Severely Impaired Extracellular Trap Formation at the Basis of Susceptibility to Infections of Hemodialysis Patients," *BMC Medicine* 20 (2022): 364, https://doi.org/10.1186/s12916-022-02564-1.

31. R. Ward and K. R. McLeish, "Soluble TNF Alpha Receptors Are Increased in Chronic Renal Insufficiency and Hemodialysis and Inhibit Neutrophil Priming by TNF Alpha," *Artificial Organs* 20 (1996): 390–395, https://doi.org/10.1111/j.1525-1594.1996.tb04521.x.

32. R. A. Ward, R. Ouseph, and K. R. McLeish, "Effects of High-Flux Hemodialysis on Oxidant Stress," *Kidney International* 63 (2003): 353–359, https://doi.org/10.1046/j.1523-1755.2003.00741.x.

33. J. W. Yoon, M. V. Pahl, and N. D. Vaziri, "Spontaneous Leukocyte Activation and Oxygen-Free Radical Generation in End-Stage Renal Disease," *Kidney International* 71 (2007): 167–172, https://doi.org/10.1038/sj.ki.5002019.

34. S. Lemesch, W. Ribitsch, G. Schilcher, et al., "Mode of Renal Replacement Therapy Determines Endotoxemia and Neutrophil Dysfunction in Chronic Kidney Disease," *Scientific Reports* 6 (2016): 34534, https://doi.org/10.1038/srep34534.

35. M. I. Muniz-Junqueira, C. B. Lopes, C. A. Magalhães, C. C. Schleicher, and J. P. Veiga, "Acute and Chronic Influence of Hemodialysis According to the Membrane Used on Phagocytic Function of Neutrophils and Monocytes and Pro-Inflammatory Cytokines Production in Chronic Renal Failure Patients," *Life Sciences* 77 (2005): 3141–3155, https://doi.org/10.1016/j.lfs.2005.03.034.

36. S. Mahajan, O. P. Kalra, K. T. Asit, G. Ahuja, and V. Kalra, "Phagocytic Polymorphonuclear Function in Patients With Progressive Uremia and the Effect of Acute Hemodialysis," *Renal Failure* 27 (2005): 357–360, https://doi.org/10.1081/JDI-200065223.

37. C. J. Porter, R. P. Burden, A. G. Morgan, I. Daniels, and J. Fletcher, "Impaired Polymorphonuclear Neutrophil Function in End-Stage Renal Failure and Its Correction by Continuous Ambulatory Peritoneal Dialysis," *Nephron* 71 (1995): 133–137, https://doi.org/10.1159/000188700.

38. J. M. Alexiewicz, M. Smogorzewski, G. Z. Fadda, and S. G. Massry, "Impaired Phagocytosis in Dialysis Patients: Studies on Mechanisms," *American Journal of Nephrology* 11 (1991): 102–111, https://doi.org/10.1159/000168284.

39. J. M. Alexiewicz, M. Smogorzewski, M. Klin, M. Akmal, and S. G. Massry, "Effect of Treatment of Hemodialysis Patients With Nifedipine on Metabolism and Function of Polymorphonuclear Leukocytes," *American Journal of Kidney Diseases* 25 (1995): 440–444, https://doi.org/10.1016/0272-6386(95)90106-x.

40. G. Banche, V. Allizond, F. Giacchino, et al., "Effect of Dialysis Membrane Biocompatibility on Polymorphonuclear Granulocyte Activity in Dialysis Patients," *Nephrology, Dialysis, Transplantation* 21 (2006): 3532–3538, https://doi.org/10.1093/ndt/gfl415.

41. A. M. Cuffini, V. Tullio, F. Giacchino, et al., "Impact of Co-Amoxiclav on Polymorphonuclear Granulocytes From Chronic Hemodialysis Patients," *American Journal of Kidney Diseases* 37 (2001): 1253–1259, https://doi.org/10.1053/ajkd.2001.24530.

42. E. M. Pappas, S. Mpournaka, P. Katopodis, et al., "The Effect of Dialysis Modality and Membrane Performance on Native Immunity in Dialysis Patients," *Pril (Makedon Akad Nauk Umet Odd Med Nauki)* 40 (2019): 25–32, https://doi.org/10.2478/prilozi-2019-0011.

43. K. Anding, P. Gross, J. M. Rost, D. Allgaier, and E. Jacobs, "The Influence of Uraemia and Haemodialysis on Neutrophil Phagocytosis and Antimicrobial Killing," *Nephrology, Dialysis, Transplantation* 18 (2003): 2067–2073, https://doi.org/10.1093/ndt/gfg330.

44. D. Scalas, G. Banche, C. Merlino, et al., "Role of Caspofungin in Restoring the Impaired Phagocyte-Dependent Innate Immunity Towards *Candida albicans* in Chronic Haemodialysis Patients," *International Journal of Antimicrobial Agents* 39 (2012): 73–76, https://doi.org/10.1016/j.ijantimicag.2011.09.015.

45. V. Tullio, A. M. Cuffini, F. Giacchino, et al., "Combined Action of Fluconazole and PMNs From Uremic Patients in Clearing Intracellular *Candida albicans*," *Journal of Chemotherapy* 15 (2003): 301–303, https://doi.org/10.1179/joc.2003.15.3.301.

46. S. Schauer, G. Stein, J. Süss, D. Falkenhagen, and W. Linss, "Phagocytosis Activity of Polymorphonuclear Cells of Normal Persons and Dialysis Patients Is Influenced by Different Dialysis Membranes," *Nephrology, Dialysis, Transplantation* 6, no. Suppl 3 (1991): 35–40.

47. P. Bartnicki, E. Majewska, M. Kowalczyk, Z. Baj, M. Banach, and J. Rysz, "Impact of Anemia Treatment With Methoxy Polyethylene Glycol-Epoetin Beta on Polymorphonuclear Cells Apoptosis in Predialysis Patients With Chronic Kidney Disease," *Pharmacological Reports* 67 (2015): 842–845, https://doi.org/10.1016/j.pharep.2015.01.014.

48. B. L. Jaber, M. C. Perianayagam, V. S. Balakrishnan, A. J. King, and B. J. Pereira, "Mechanisms of Neutrophil Apoptosis in Uremia and Relevance of the Fas (APO-1, CD95)/Fas Ligand System," *Journal of Leukocyte Biology* 69 (2001): 1006–1012.

49. N. Zahran, A. Sayed, I. William, O. Mahmoud, O. Sabry, and M. Rafaat, "Neutrophil Apoptosis: Impact of Granulocyte Macrophage Colony Stimulating Factor on Cell Survival and Viability in Chronic Kidney Disease and Hemodialysis Patients," *Archives of Medical Science* 9 (2013): 984–989, https://doi.org/10.5114/aoms.2013.39789.

50. N. Nahar, H. Shah, J. Siu, et al., "Dialysis Membrane-Induced Neutrophil Apoptosis Is Mediated Through Free Radicals," *Clinical Nephrology* 56 (2001): 52–59.

51. G. Cohen, J. Raupachova, T. Wimmer, R. Deicher, and W. H. Hörl, "The Uraemic Retention Solute Para-Hydroxy-Hippuric Acid Attenuates Apoptosis of Polymorphonuclear Leukocytes From Healthy Subjects but Not From Haemodialysis Patients," *Nephrology, Dialysis, Transplantation* 23 (2008): 2512–2519, https://doi.org/10.1093/ndt/gfn098.

52. L. Dou, P. Brunet, F. Dignat-George, J. Sampol, and Y. Berland, "Effect of Uremia and Hemodialysis on Soluble L-Selectin and Leukocyte Surface CD11b and L-Selectin," *American Journal of Kidney Diseases* 31 (1998): 67–73, https://doi.org/10.1053/ajkd.1998.v31.pm9428454.

53. J. Olsson, T. A. Jacobson, J. M. Paulsson, et al., "Expression of Neutrophil SOD2 Is Reduced After Lipopolysaccharide Stimulation: A

17481716, 2025, 6, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/aphw.70057 by Jane Sophie Lauxen - Rwth Aachen University Zentraler Rechnungseingang , Wiley Online Library on [25/05/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Potential Cause of Neutrophil Dysfunction in Chronic Kidney Disease," *Nephrology, Dialysis, Transplantation* 26 (2011): 2195–2201, https://doi.org/10.1093/ndt/gfq673.

54. C. Wallquist, L. Mansouri, M. Norrback, et al., "Associations of Fibroblast Growth Factor 23 With Markers of Inflammation and Leukocyte Transmigration in Chronic Kidney Disease," *Nephron* 138 (2018): 287–295, https://doi.org/10.1159/000485472.

55. D. M. Eldewi, A. M. Alhabibi, H. M. E. El Sayek, et al., "Expression Levels of Complement Regulatory Proteins (CD35, CD55 and CD59) on Peripheral Blood Cells of Patients With Chronic Kidney Disease," *International Journal of General Medicine* 12 (2019): 343–351, https://doi.org/10.2147/ijgm.S216989.

56. Z. Aljadi, L. Mansouri, A. Nopp, et al., "Activation of Basophils Is a New and Sensitive Marker of Biocompatibility in Hemodialysis," *Artificial Organs* 38 (2014): 945–953, https://doi.org/10.1111/aor.12297.

57. R. Pereira, E. Costa, M. Gonçalves, et al., "Neutrophil and Monocyte Activation in Chronic Kidney Disease Patients Under Hemodialysis and Its Relationship With Resistance to Recombinant Human Erythropoietin and to the Hemodialysis Procedure," *Hemodialysis International* 14 (2010): 295–301, https://doi.org/10.1111/j.1542-4758.2010.00450.x.

58. E. Costa, S. Rocha, P. Rocha-Pereira, et al., "Neutrophil Activation and Resistance to Recombinant Human Erythropoietin Therapy in Hemodialysis Patients," *American Journal of Kidney Diseases* 28, no. 6 (2008): 935–940, https://doi.org/10.1159/000142147.

59. E. Khatib-Massalha, R. Michelis, B. Trabelcy, et al., "Free Circulating Active Elastase Contributes to Chronic Inflammation in Patients on Hemodialysis," *American Journal of Physiology. Renal Physiology* 314 (2018): F203–f209, https://doi.org/10.1152/ajprenal.00070.2017.

60. C. Capeillere-Blandin, V. Gausson, A. T. Nguyen, B. Descamps-Latscha, T. Drueke, and V. Witko-Sarsat, "Respective Role of Uraemic Toxins and Myeloperoxidase in the Uraemic State," *Nephrology, Dialysis, Transplantation* 21 (2006): 1555–1563, https://doi.org/10.1093/ndt/gfl007.

61. M. S. Tsai, H. M. Shaw, Y. J. Li, M. T. Lin, W. T. Lee, and K. S. Chan, "Myeloperoxidase in Chronic Kidney Disease: Role of Visceral Fat," *Nephrology* 19 (2014): 136–142, https://doi.org/10.1111/nep.12187.

62. R. Deicher, M. Exner, G. Cohen, M. Haag-Weber, and W. H. Hörl, "Neutrophil Beta(2)-Microglobulin and Lactoferrin Content in Renal Failure Patients," *American Journal of Kidney Diseases* 35 (2000): 1117–1126, https://doi.org/10.1016/s0272-6386(00)70049-7.

63. J. C. Jeong, J. E. Kim, J. Y. Gu, et al., "Significance of the DNA-Histone Complex Level as a Predictor of Major Adverse Cardiovascular Events in Hemodialysis Patients: The Effect of Uremic Toxin on DNA-Histone Complex Formation," *Blood Purification* 41 (2016): 64–71, https://doi.org/10.1159/000440974.

64. J. K. Kim, M. J. Park, H. W. Lee, et al., "The Relationship Between Autophagy, Increased Neutrophil Extracellular Traps Formation and Endothelial Dysfunction in Chronic Kidney Disease," *Clinical Immunology* 197 (2018): 189–197, https://doi.org/10.1016/j.clim.2018.10.003.

65. C. Vega-Roman, C. Leal-Cortes, E. Portilla-de Buen, et al., "Impact of Transplantation on Neutrophil Extracellular Trap Formation in Patients With End-Stage Renal Disease: A Single-Center, Prospective Cohort Study," *Medicine* 100 (2021): e26595, https://doi.org/10.1097/md.0000000000026595.

66. S. Grazioli and J. Pugin, "Mitochondrial Damage-Associated Molecular Patterns: From Inflammatory Signaling to Human Diseases," *Frontiers in Immunology* 9 (2018): 832, https://doi.org/10.3389/fimmu.2018.00832.

67. M. G. Betjes, "Immune Cell Dysfunction and Inflammation in End-Stage Renal Disease," *Nature Reviews Nephrology* 9 (2013): 255–265, https://doi.org/10.1038/nrneph.2013.44.

68. T. Ebert, O. Neytchev, A. Witasp, K. Kublickiene, P. Stenvinkel, and P. G. Shiels, "Inflammation and Oxidative Stress in Chronic Kidney Disease and Dialysis Patients," *Antioxidants & Redox Signaling* 35 (2021): 1426–1448, https://doi.org/10.1089/ars.2020.8184.

69. R. Vanholder and S. Ringoir, "Polymorphonuclear Cell Function and Infection in Dialysis," *Kidney International. Supplement* 38 (1992): S91–S95.

70. G. Cohen, M. Haag-Weber, and W. H. Hörl, "Immune Dysfunction in Uremia," *Kidney International. Supplement* 62 (1997): S79–S82.

71. E. Papachristoforou, V. Lambadiari, E. Maratou, and K. Makrilakis, "Association of Glycemic Indices (Hyperglycemia, Glucose Variability, and Hypoglycemia) With Oxidative Stress and Diabetic Complications," *Journal of Diabetes Research* 2020 (2020): 7489795, https://doi.org/10.1155/2020/7489795.

72. S. L. Wong, M. Demers, K. Martinod, et al., "Diabetes Primes Neutrophils to Undergo NETosis, Which Impairs Wound Healing," *Nature Medicine* 21 (2015): 815–819, https://doi.org/10.1038/nm.3887.

73. R. Vanholder, W. Van Biesen, and S. Ringoir, "Contributing Factors to the Inhibition of Phagocytosis in Hemodialyzed Patients," *Kidney International* 44 (1993): 208–214, https://doi.org/10.1038/ki.1993.232.

74. L. P. Roma, M. Deponte, J. Riemer, and B. Morgan, "Mechanisms and Applications of Redox-Sensitive Green Fluorescent Protein-Based Hydrogen Peroxide Probes," *Antioxidants & Redox Signaling* 29 (2018): 552–568, https://doi.org/10.1089/ars.2017.7449.

75. M. Schwarzländer, T. P. Dick, A. J. Meyer, and B. Morgan, "Dissecting Redox Biology Using Fluorescent Protein Sensors," *Antioxidants & Redox Signaling* 24 (2016): 680–712, https://doi.org/10.1089/ars.2015.6266.

76. L. P. Roma and J. C. Jonas, "Nutrient Metabolism, Subcellular Redox State, and Oxidative Stress in Pancreatic Islets and β-Cells," *Journal of Molecular Biology* 432 (2020): 1461–1493, https://doi.org/10.1016/j.jmb.2019.10.012.

77. H. Sies, V. V. Belousov, N. S. Chandel, et al., "Defining Roles of Specific Reactive Oxygen Species (ROS) in Cell Biology and Physiology," *Nature Reviews. Molecular Cell Biology* 23 (2022): 499–515, https://doi.org/10.1038/s41580-022-00456-z.

78. C. Silvestre-Roig, A. Hidalgo, and O. Soehnlein, "Neutrophil Heterogeneity: Implications for Homeostasis and Pathogenesis," *Blood* 127 (2016): 2173–2181, https://doi.org/10.1182/blood-2016-01-688887.

79. M. Palomino-Segura, J. Sicilia, I. Ballesteros, and A. Hidalgo, "Strategies of Neutrophil Diversification," *Nature Immunology* 24 (2023): 575–584, https://doi.org/10.1038/s41590-023-01452-x.

80. C. Baaten, S. Vondenhoff, and H. Noels, "Endothelial Cell Dysfunction and Increased Cardiovascular Risk in Patients With Chronic Kidney Disease," *Circulation Research* 132 (2023): 970–992, https://doi.org/10.1161/circresaha.123.321752.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.

17481716, 2025, 6, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/apha.70057 by Jane Sophie Lauxen - Rwth Aachen University Zentraler Rechnungseingang , Wiley Online Library on [25/05/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Neutrophil function in patients with chronic kidney disease: a systematic review and meta-analysis

By Lauxen et al.

Table of contents

Supplementary results with references p. 2
Supplementary tables p. 9
Supplementary figures p.19

Supplementary results

Neutrophil functional changes in CKD: a systematic review

Phagocytosis. Results on phagocytosis activity in CKD were highly variable, also within the same study (n=19 studies; n=13 decreased[1-13]; n=13 unaltered[3, 4, 6, 10-19] in CKD vs. healthy controls; **Suppl. Table 2**). The results were partly dependent on whether CKD patients were on dialysis as well as on the dialysis type: an unaltered phagocytic capacity was reported in four of six studies on non-dialysis CKD patients (n=4 unaltered[3, 4, 6, 12]; vs. n=2 reduced phagocytosis[10, 13]) and in all studies on PD patients (n=5[3, 4, 6, 10, 13]) (**Table 1**). In contrast, of the 15 studies analyzing HD patients, 67% (10 of 15) detected reduced phagocytosis capacity vs. controls[1-8, 11, 12] and one study detected reduced or unaltered phagocytosis dependent on the dialysis membrane used[11], with the other five studies not identifying differences[14-17, 19] (**Table 1; Suppl. Table 3**). A similar conclusion holds when only accounting the studies that simultaneously compared non-dialysis and dialysis patients (HD or PD) to controls (**Suppl. Table 3**). In conclusion, neutrophil phagocytic capacity was mostly found unaltered in non-dialysis CKD patients and CKD patients on PD, whereas it was reduced in 67% of studies in dialysis patients on HD.

Apoptosis. Also findings on neutrophil apoptosis were partly dependent on whether non-dialysis CKD patients or patients on dialysis were analyzed: All five studies analyzing neutrophils of non-dialysis patients reported increased apoptosis compared to healthy controls[4, 20-23]. Instead, of the eight studies analyzing neutrophils of dialysis patients, only three reported on increased apoptosis in HD[22-24] or PD patients[22], whereas the other (n=5) did not detect differences in either HD[4, 21, 25-27] or PD patients[4, 21] vs. controls (**Table 1**, **Suppl. Tables 2 and 4**). Summarized, neutrophil apoptosis was increased consistently in non-dialysis CKD patients, but only in subset of studies in CKD patients on HD or PD.

Surface activation markers. *(Basal)* In unstimulated conditions, 10 of 14 studies (71%) reported an increased surface activation marker expression in CKD patients vs. healthy controls (e.g. for

CD11b and CD18)[8, 23, 28-35] - although not always of all investigated markers – and/or a reduction of surface CD62L, which is known to be shed upon neutrophil activation[32, 35] (**Suppl. Tables 2 and 5**). For CD11b, CD18, CD66b and CD88 as the most frequently studied activation markers, four of five studies analyzing non-dialysis CKD patients found increased expression compared to healthy controls (n=4[23, 31, 32, 34]), while one found reduced expression (n=1[36]). In contrast, HD patients were reported to have one or more of these four activation markers increased only in 50% of the studies (increased: n=4[8, 23, 29, 33]; unaltered: n=4[30, 32, 37, 38]) (**Table 1, Suppl. Table 5B**). When comparing CD62L before vs. after a hemodialysis session, CD62L was reduced after dialysis, indicating neutrophil activation during the dialysis session[35]. One study identified a decreased surface expression of the chemokine receptor CXCR1 in dialysis patients compared to controls[38], which is reduced upon neutrophil activation. Two studies on HD patients could not detect any investigated activation marker (CD11b, TLR4/9, CD88) to be altered in basal conditions[37, 39] (**Suppl. Table 5B**).

(Stimulated) Only four studies examined the impact of CKD on surface activation markers of stimulated neutrophils. Three studies (2x non-dialysis CKD; 1x not-specified CKD cohort) showed a stronger CD11b increase in CKD patients compared to healthy controls (e.g. upon stimulation with phorbol myristate acetate (PMA), N-formyl-methionyl-leucyl-phenylalanine (fMLP) or lipopolysaccharides (LPS))[28, 34, 40], although other markers as CD62L or CD11a remained unaltered (**Suppl. Table 5B**). One study in HD patients (IL8-stimulation) could not detect a difference in CD11b expression compared to healthy controls[37] (**Table 1**, **Suppl. Table 5B**).

In summary, analysis of surface activation markers revealed generally increased neutrophil activation in non-dialysis CKD patients in both basal and stimulated conditions, but only in around half of studies on unstimulated neutrophils from HD patients. Insufficient data are yet available on stimulated neutrophils in HD patients as well as on neutrophils from PD patients.

Degranulation. All studies observed an increase in at least one of the neutrophil degranulation readouts in either blood or isolated neutrophils in CKD patients compared to controls (n=8: 7x HD patients; 1x unspecified CKD stage)[8, 41-47]. These readouts included (i) increased levels of neutrophil elastase, MPO, lactoferrin or histone-DNA in blood, (ii) reduced basal levels of MPO or

neutrophil elastase in neutrophils, or (iii) an increased MPO or neutrophil elastase release upon neutrophil stimulation (**Suppl. Tables 2 and 6**). Only one study compared non-dialysis CKD and HD patients, revealing increased plasma MPO levels only in HD patients[44] (**Suppl. Table 6**). In summary, neutrophil degranulation was mainly found to be increased in HD patients (**Figure 2)**, with no comparison possible to PD patients or non-dialysis CKD patients due to a lack of sufficient data.

NET formation. NET formation was predominantly assessed by quantification of nucleic acid by Sytox-Green-fluorescence in isolated neutrophils. Four studies analyzed NET formation in neutrophils from CKD patients (3x HD; 1x combining patients with HD, PD and non-dialysis CKD), however, with variable results (**Table 1, Suppl. Tables 2 and 7**): *(Basal)* Using resting neutrophils, two studies observed an increased NET formation vs. healthy controls[30, 48] whereas the other two studies did not observe differences[8, 49]. *(Stimulated)* Upon stimulation with PMA or calcium ionophore (A23187), two studies showed no alterations between dialysis-patients and controls[30, 49], one study showed increased[48] and one decreased NET formation[8]. In summary, highly variable data were observed for NET formation by neutrophils from HD patients, with no comparison possible to PD patients or non-dialysis CKD patients due to a lack of data.

ROS production. Neutrophils produce ROS via the NADPH-oxidase (NOX2) at the plasma membrane (extracellular ROS) or in intracellular compartments (intracellular ROS)[50]. Findings on neutrophil ROS production in CKD patients varied strongly: *(Basal)* From 13 studies that measured basal ROS levels in neutrophils of CKD patients, seven found increased ROS compared to healthy controls[3, 15, 25, 27, 30, 33, 44], while seven found the parameter unaltered[3, 4, 18, 43, 44, 51, 52] with sometimes variable results obtained within the same study dependent on the patient cohort[3, 44]; only one study found decreased basal ROS production in neutrophils from CKD patients[53] (**Suppl. Table 2**). When discriminating between non-dialysis CKD, PD and HD patients, an increased basal ROS production was observed in 8 of 12 studies on HD patients, whereas no changes were detected in non-dialysis CKD patients (n=4) or PD patients (n=2) (**Table 1, suppl. Table 8**).

(Stimulated) A high data variability was also observed for ROS production in stimulated neutrophils of CKD patients (n=20 publications), even within the same study depending on the patient cohort or stimulus analyzed, with respectively nine, seven and ten studies observing decreased[8, 15, 22, 53-58], unaltered[3, 4, 10, 14, 18, 27, 54] or increased[3, 4, 18, 22, 27, 29, 51, 52, 59, 60] ROS production in CKD compared to healthy controls (**Suppl. Table 2**). Data remained highly variable even when discriminating between non-dialysis, PD and HD-patients (**Table 1, suppl. Table 8**). In non-dialysis CKD patients (n=7 publications), stimulated ROS production was observed to be increased[18, 22, 59], decreased[10, 22, 58] or unaltered[3, 4, 18, 58] dependent on the stimulus analyzed (**Table 1**). Similarly, a high variability was seen in HD and PD patients (**Table 1**).

Overall, a high data variability was observed when summarizing neutrophil ROS production in CKD patients, urging a closer look into data variability and potential subgroups using a meta-analysis.

Supplemental References

1. Alexiewicz, J.M., et al., *Effect of treatment of hemodialysis patients with nifedipine on metabolism and function of polymorphonuclear leukocytes.* Am J Kidney Dis, 1995. **25**(3): p. 440-4.
2. Cuffini, A.M., et al., *Impact of co-amoxiclav on polymorphonuclear granulocytes from chronic hemodialysis patients.* Am J Kidney Dis, 2001. **37**(6): p. 1253-9.
3. Lemesch, S., et al., *Mode of renal replacement therapy determines endotoxemia and neutrophil dysfunction in chronic kidney disease.* Sci Rep, 2016. **6**: p. 34534.
4. Sardenberg, C., et al., *Effects of uraemia and dialysis modality on polymorphonuclear cell apoptosis and function.* Nephrol Dial Transplant, 2006. **21**(1): p. 160-5.
5. Alexiewicz, J.M., et al., *Impaired phagocytosis in dialysis patients: studies on mechanisms.* Am J Nephrol, 1991. **11**(2): p. 102-11.
6. Muniz-Junqueira, M.I., et al., *Acute and chronic influence of hemodialysis according to the membrane used on phagocytic function of neutrophils and monocytes and pro-inflammatory cytokines production in chronic renal failure patients.* Life Sci, 2005. **77**(25): p. 3141-55.
7. Pappas, E.M., et al., *The Effect of Dialysis Modality and Membrane Performance on Native Immunity in Dialysis Patients.* Pril (Makedon Akad Nauk Umet Odd Med Nauki), 2019. **40**(2): p. 25-32.
8. Talal, S., et al., *Neutrophil degranulation and severely impaired extracellular trap formation at the basis of susceptibility to infections of hemodialysis patients.* BMC Med, 2022. **20**(1): p. 364.
9. Peng, D.Z., et al., *Relationship between leptin and chronic inflammatory state in uremic patients.* European Review for Medical and Pharmacological Sciences, 2014. **18**(19): p. 2882-2885.
10. Porter, C.J., et al., *Impaired bacterial killing and hydrogen peroxide production by polymorphonuclear neutrophils in end-stage renal failure.* Nephron, 1997. **77**(4): p. 479-81.

11. Banche, G., et al., *Effect of dialysis membrane biocompatibility on polymorphonuclear granulocyte activity in dialysis patients.* Nephrology Dialysis Transplantation, 2006. **21**(12): p. 3532-3538.
12. Mahajan, S., et al., *Phagocytic polymorphonuclear function in patients with progressive uremia and the effect of acute hemodialysis.* Ren Fail, 2005. **27**(4): p. 357-60.
13. Porter, C.J., et al., *Impaired polymorphonuclear neutrophil function in end-stage renal failure and its correction by continuous ambulatory peritoneal dialysis.* Nephron, 1995. **71**(2): p. 133-7.
14. Anding, K., et al., *The influence of uraemia and haemodialysis on neutrophil phagocytosis and antimicrobial killing.* Nephrol Dial Transplant, 2003. **18**(10): p. 2067-73.
15. Patruta, S.I., et al., *Neutrophil impairment associated with iron therapy in hemodialysis patients with functional iron deficiency.* J Am Soc Nephrol, 1998. **9**(4): p. 655-63.
16. Scalas, D., et al., *Role of caspofungin in restoring the impaired phagocyte-dependent innate immunity towards Candida albicans in chronic haemodialysis patients.* Int J Antimicrob Agents, 2012. **39**(1): p. 73-6.
17. Tullio, V., et al., *Combined action of fluconazole and PMNs from uremic patients in clearing intracellular Candida albicans.* J Chemother, 2003. **15**(3): p. 301-3.
18. Ward, R.A. and K.R. McLeish, *Polymorphonuclear leukocyte oxidative burst is enhanced in patients with chronic renal insufficiency.* J Am Soc Nephrol, 1995. **5**(9): p. 1697-702.
19. Schauer, S., et al., *Phagocytosis activity of polymorphonuclear cells of normal persons and dialysis patients is influenced by different dialysis membranes.* Nephrol Dial Transplant, 1991. **6 Suppl 3**: p. 35-40.
20. Bartnicki, P., et al., *Impact of anemia treatment with methoxy polyethylene glycol-epoetin beta on polymorphonuclear cells apoptosis in predialysis patients with chronic kidney disease.* Pharmacol Rep, 2015. **67**(5): p. 842-5.
21. Jaber, B.L., et al., *Mechanisms of neutrophil apoptosis in uremia and relevance of the Fas (APO-1, CD95)/Fas ligand system.* J Leukoc Biol, 2001. **69**(6): p. 1006-12.
22. Sela, S., et al., *Primed peripheral polymorphonuclear leukocyte: a culprit underlying chronic low-grade inflammation and systemic oxidative stress in chronic kidney disease.* J Am Soc Nephrol, 2005. **16**(8): p. 2431-8.
23. Zahran, N., et al., *Neutrophil apoptosis: impact of granulocyte macrophage colony stimulating factor on cell survival and viability in chronic kidney disease and hemodialysis patients.* Arch Med Sci, 2013. **9**(6): p. 984-9.
24. Nahar, N., et al., *Dialysis membrane-induced neutrophil apoptosis is mediated through free radicals.* Clin Nephrol, 2001. **56**(1): p. 52-9.
25. Perianayagam, M.C., et al., *Quantification of Bax and Bcl2 in polymorphonuclear leukocytes from haemodialysis patients: relation to hydrogen peroxide.* Eur J Clin Invest, 2003. **33**(10): p. 905-11.
26. Cohen, G., et al., *The uraemic retention solute para-hydroxy-hippuric acid attenuates apoptosis of polymorphonuclear leukocytes from healthy subjects but not from haemodialysis patients.* Nephrol Dial Transplant, 2008. **23**(8): p. 2512-9.
27. Guo, D., et al., *Impact of iron dextran on polymorphonuclear cell function among hemodialysis patients.* Clin Nephrol, 2002. **58**(2): p. 134-42.
28. Caimi, G., et al., *Polymorphonuclear leukocyte integrins in chronic renal failure.* Clin Hemorheol Microcirc, 2005. **32**(1): p. 43-9.
29. Cohen-Mazor, M., et al., *Are primed polymorphonuclear leukocytes contributors to the high heparanase levels in hemodialysis patients?* Am J Physiol Heart Circ Physiol, 2008. **294**(2): p. H651-8.
30. Kim, J.K., et al., *Increased Neutrophil Extracellular Trap Formation in Uremia Is Associated with Chronic Inflammation and Prevalent Coronary Artery Disease.* J Immunol Res, 2017. **2017**: p. 8415179.
31. Olsson, J., et al., *Expression of neutrophil SOD2 is reduced after lipopolysaccharide stimulation: a potential cause of neutrophil dysfunction in chronic kidney disease.* Nephrol Dial Transplant, 2011. **26**(7): p. 2195-201.
32. Dou, L., et al., *Effect of uremia and hemodialysis on soluble L-selectin and leukocyte surface CD11b and L-selectin.* Am J Kidney Dis, 1998. **31**(1): p. 67-73.

33. Yoon, J.W., M.V. Pahl, and N.D. Vaziri, *Spontaneous leukocyte activation and oxygen-free radical generation in end-stage renal disease.* Kidney Int, 2007. **71**(2): p. 167-72.
34. Wallquist, C., et al., *Associations of Fibroblast Growth Factor 23 with Markers of Inflammation and Leukocyte Transmigration in Chronic Kidney Disease.* Nephron, 2018. **138**(4): p. 287-295.
35. Hassoba, H., M. Mahmoud, and A. El-Gohary, *Alterations in neutrophil surface expression of L-selectin (CD62L) and soluble L-selectin (sL-selectin) in hemodialysis patients: relation to HCV.* Egypt J Immunol, 2007. **14**(2): p. 101-10.
36. Eldewi, D.M., et al., *Expression levels of complement regulatory proteins (CD35, CD55 and CD59) on peripheral blood cells of patients with chronic kidney disease.* International Journal of General Medicine, 2019. **12**: p. 343-351.
37. Aljadi, Z., et al., *Activation of basophils is a new and sensitive marker of biocompatibility in hemodialysis.* Artif Organs, 2014. **38**(11): p. 945-53.
38. Pereira, R., et al., *Neutrophil and monocyte activation in chronic kidney disease patients under hemodialysis and its relationship with resistance to recombinant human erythropoietin and to the hemodialysis procedure.* Hemodial Int, 2010. **14**(3): p. 295-301.
39. Baj, Z., et al., *Basic inflammatory indices and chosen neutrophil receptors expression in chronic haemodialysed patients.* Central European Journal of Immunology, 2018. **43**(2): p. 168-173.
40. Olsson, J., et al., *Expression of neutrophil SOD2 is reduced after lipopolysaccharide stimulation: a potential cause of neutrophil dysfunction in chronic kidney disease.* Nephrology Dialysis Transplantation, 2011. **26**(7): p. 2195-2201.
41. Costa, E., et al., *Neutrophil activation and resistance to recombinant human erythropoietin therapy in hemodialysis patients.* Am J Nephrol, 2008. **28**(6): p. 935-40.
42. Khatib-Massalha, E., et al., *Free circulating active elastase contributes to chronic inflammation in patients on hemodialysis.* Am J Physiol Renal Physiol, 2018. **314**(2): p. F203-f209.
43. Otaki, Y., et al., *Defective regulation of iron transporters leading to iron excess in the polymorphonuclear leukocytes of patients on maintenance hemodialysis.* Am J Kidney Dis, 2004. **43**(6): p. 1030-9.
44. Capeillere-Blandin, C., et al., *Respective role of uraemic toxins and myeloperoxidase in the uraemic state.* Nephrology Dialysis Transplantation, 2006. **21**(6): p. 1555-1563.
45. Tsai, M.S., et al., *Myeloperoxidase in chronic kidney disease: Role of visceral fat.* Nephrology, 2014. **19**(3): p. 136-142.
46. Deicher, R., et al., *Neutrophil beta(2)-microglobulin and lactoferrin content in renal failure patients.* Am J Kidney Dis, 2000. **35**(6): p. 1117-26.
47. Jeong, J.C., et al., *Significance of the DNA-Histone Complex Level as a Predictor of Major Adverse Cardiovascular Events in Hemodialysis Patients: The Effect of Uremic Toxin on DNA-Histone Complex Formation.* Blood Purif, 2016. **41**(1-3): p. 64-71.
48. Kim, J.K., et al., *The relationship between autophagy, increased neutrophil extracellular traps formation and endothelial dysfunction in chronic kidney disease.* Clin Immunol, 2018. **197**: p. 189-197.
49. Vega-Roman, C., et al., *Impact of transplantation on neutrophil extracellular trap formation in patients with end-stage renal disease: A single-center, prospective cohort study.* Medicine (Baltimore), 2021. **100**(27): p. e26595.
50. Dahlgren, C., A. Karlsson, and J. Bylund, *Intracellular Neutrophil Oxidants: From Laboratory Curiosity to Clinical Reality.* J Immunol, 2019. **202**(11): p. 3127-3134.
51. Ward, R.A., R. Ouseph, and K.R. McLeish, *Effects of high-flux hemodialysis on oxidant stress.* Kidney Int, 2003. **63**(1): p. 353-9.
52. Paul, J.L., et al., *Influence of uremia on polymorphonuclear leukocytes oxidative metabolism in end-stage renal disease and dialyzed patients.* Nephron, 1991. **57**(4): p. 428-32.
53. Kiersztejn, M., et al., *Decreased O2 consumption by PMNL from humans and rats with CRF: role of secondary hyperparathyroidism.* Kidney Int, 1992. **42**(3): p. 602-9.
54. Rao, M., et al., *Dialyzer membrane type and reuse practice influence polymorphonuclear leukocyte function in hemodialysis patients.* Kidney Int, 2004. **65**(2): p. 682-91.

55. Cohen, G., et al., *Effect of leptin on polymorphonuclear leucocyte functions in healthy subjects and haemodialysis patients.* Nephrol Dial Transplant, 2011. **26**(7): p. 2271-81.
56. Cohen-Hagai, K., et al., *Malnutrition and inflammation in hemodialysis patients: Comparative evaluation of neutrophil reactive oxygen formation.* Nutrition, 2020. **78**: p. 110793.
57. Dobos, G.J., et al., *Neutrophil dysfunction in end-stage renal failure: reduced response to priming by C5a.* Clin Investig, 1994. **72**(5): p. 353-7.
58. Ward, R. and K.R. McLeish, *Soluble TNF alpha receptors are increased in chronic renal insufficiency and hemodialysis and inhibit neutrophil priming by TNF alpha.* Artif Organs, 1996. **20**(5): p. 390-5.
59. Haynes, A.P., et al., *Abnormal cytoplasmic pH regulation during activation in uremic neutrophils.* Kidney Int, 1992. **42**(3): p. 690-9.
60. Kristal, B., et al., *Interaction between erythropoietin and peripheral polymorphonuclear leukocytes in hemodialysis patients.* Nephron, 1999. **81**(4): p. 406-13.
61. Ward, R.A. and K.R. McLeish, *Soluble TNF alpha receptors are increased in chronic renal insufficiency and hemodialysis and inhibit neutrophil priming by TNF alpha.* Artificial Organs, 1996. **20**(5): p. 390-395.

Supplementary tables

Suppl. Table 1. Search strategy, in- and exclusion criteria for the identification of studies investigating neutrophil function in CKD patients. This study was performed according to the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guidelines (not registered).

SYSTEMATIC REVIEW: Search strategy
PubMed: ((("neutrophils"[MeSH Terms]) OR ("granulocytes"[MeSH Terms])) AND (renal insufficiency, chronic[MeSH Terms])) AND (("1990"[Date - Publication] : "3000"[Date - Publication]))
Web of Science: ((AB=(neutrophils)) OR (AB=(granulocytes))) AND ((TS=(“chronic renal insufficiency”)) OR (TS=(CKD)) OR (TS=("chronic kidney disease”))) AND PY=(1990-2023) With “exact search”
SYSTEMATIC REVIEW: In- and exclusion criteria during title and abstract screening (first stage)
Inclusion criteria
- if neutrophil function in blood of patients with CKD was assessed in comparison to that of healthy volunteers
Exclusion criteria
- if written in another language than English - if the work was non-original or only conference abstracts were available - if being a meta-analysis or systemic review - if being an ongoing clinical trial, study protocol or case report - if exclusively dealing with animal studies - if only specific underlying pathologies of CKD were investigated (e.g. lupus nephritis) or no CKD focus was included (no CKD or too specific CKD pathology) - if neutrophils were not investigated
SYSTEMATIC REVIEW: Additional exclusion criteria during full text assessment (second stage)
- if the full text could not be accessed - if using patient material other than blood - if only including transplanted patients - if only measuring neutrophil-to-lymphocyte-ratio or neutrophil numbers - if lacking a healthy control group -if they used non-adult control group vs. adult experimental group - if only specific underlying pathologies of CKD were investigated (e.g. lupus nephritis, cancer associated) or no CKD focus was included (no CKD or too specific CKD pathology)
META-ANALYSIS of ROS production: Additional exclusion criteria (third stage)
Inclusion criteria
- if studies analyzed neutrophil ROS production in CKD patients on dialysis compared to healthy controls
Exclusion criteria

- if studies used an indirect measurement of ROS like oxygen consumption via Clarke electrode or measurement of CO_2 production via radiolabeled glucose (incomparable assay of ROS analysis used)
- resulting data only given as median
- patients reported to be newly started on dialysis
- studies that did not fulfill the criterium of certainty in the body of evidence for ROS analysis
    - number of included controls/patients unclear (missing experimental details)
    - controls and patients not clearly defined
    - study population not clearly defined

Suppl. Table 2. Summary of overall outcome of neutrophil activity parameters in patients with chronic kidney disease compared to healthy controls. Studies are counted towards a certain category (decreased/unaltered/increased) if one of their included datasets showed the respective outcome, and may therefore be included in different categories; the sum of all categories (decreased/unaltered/increased) can therefore exceed the total number of studies per readout. *fMLP, N-formyl-methionyl-leucyl-phenylalanine; LPS, lipopolysaccharides; NETs, neutrophil extracellular traps; PAMPS, Pathogen-associated molecular patterns; PMA, phorbol-12-myristate-13-acetate; ROS, reactive oxygen species.*

Parameters	Nr. of studies	Decreased		Unaltered		Increased	
		Nr.	Ref.	Nr	Ref.	Nr.	Ref.
Phagocytosis	19	13	[1-13]	13	[3, 4, 6, 10-19]	0	
Apoptosis	9	0	-	5	[4, 21, 25-27]	6	[4, 20-24]
Surface activation markers – Basal	14	3	*(Including studies with increased CD62L, reflecting decreased neutrophil activation)* [35, 36, 38]	8	[8, 28, 30, 32, 34, 37-39]	10	*(Including studies with decreased CD62L, reflecting increased neutrophil activation)* [8, 23, 28-35]
Surface activation markers – Stimulated neutrophils	4	0	-	3	[28, 34, 37]	3	[28, 31, 34]
Degranulation	8	1	[43]	4	[41, 44, 46, 47]	8	[8, 41-47]
NET formation-basal	4	0		2	[8, 49]	2	[30, 48]
NET formation-stimulated neutrophils	4	1	[8]	2	[30, 49]	1	[48]
ROS production-Basal	13	1	[53]	7	[3, 4, 18, 43, 44, 51, 52]	7	[3, 15, 25, 27, 30, 33, 44]
ROS production-Stimulated by:	20	9		7		10	
PAMPs (E.Coli/LPS, S. aureus, C. albicans, Zymosan)	11	4	[8, 22, 55, 56]	4	[3, 4, 10, 14]	5	[3, 4, 27, 51, 52]
PMA	12	5	[8, 15, 54-56]	3	[4, 18, 27]	4	[22, 29, 52, 60]
fMLP	7	2	[61](fMLP+TNFα) [53]	2	[4, 54]	4	[4, 18, 27, 59]
Complement	1	1	[57](C5a +FLPEP)	0	-	0	-

Author	Year	PMID	CKD Stage	Dialysis type (for CKD5 patients)	Phagocytotic activity: target	Method used	Author conclusions	Increased/ decreased/ unaltered *phagocytosis* (CKD patients vs. healthy controls)
						with E. coli LPS		
Anding et al.	2003	13679482	5	HD	*C.albicans*	Analysis of uptake of labeled C.albicans by flow cytometry	Slightly lower, but not statistically significant	Unaltered
Banche et al.	2006	16935897	5	HD	*K. pneumoniae*	Analysis of uptake of labeled K. pneumoniae by spectrophotometry	Compared to healthy controls, unaltered in HD when using CL-E or PS membrane but decreased when using SMC dialysis membrane	Decreased in HD when using SMC dialysis membrane Unaltered in HD when using a CL-E and PS membrane
Cuffini et al.	2001	11382696	5	HD	*K.pneumoniae*	Analysis of uptake of labeled K.pneumoniae by spectrophotometry	Lower phagocytosis after 30 and 90 min bacterial stimulation	Decreased
Lemesch et al.	2016	27698480	3 to 5	HD and PD	*E.coli*	Analysis of uptake of labeled E.coli by flow cytometry	Significantly lower in patients undergoing HD, but not in PD. No differences in non-dialysis CKD	Unaltered in non-dialysis CKD and PD Decreased in HD
Mahajan et al.	2005	16060119	2 to 5	HD	*IgG coated sheep erythrocytes*	Analysis of uptake of IgG-coated sheep erythrocytes by spectrophotometry	Compared to controls, significantly reduced in patients undergoing HD but not in non-dialysis CKD. Significantly reduced before compared to after HD session.	Unaltered in non-dialysis CKD Decreased in HD
Muniz-Junqueira et al.	2005	16005905	Non-dialysis not specified and 5	HD and PD	*S.cerevisiae*	Analysis of uptake of labeled S. cerevisiae by spectrophotometry	Significantly lower in patients undergoing HD, but not in PD. No differences in non-dialysis CKD	Unaltered in non-dialysis CKD and PD Decreased in HD
Pappas et al.	2019	31605588	5	HD	*E.Coli*	Analysis of uptake of labeled E.Coli by flow cytometry	Significantly impaired	Decreased
Patruta et al.	1998	9555668	5	HD	*E.coli*	Killing capacity by fluorescence microscopy	Lower, but not statistically significant	Unaltered
Peng et al.	2014	25339482	Not specified	Not specified	*Not specified yeasts*	Killing capacity by microscopy	Significantly impaired	Decreased
Porter et al.	1995	8569942	Non-dialysis CKD 5	PD	*S. epidermidis*	Analysis of uptake of labeled S. epidermidis by spectrophotometry	Decreased in non-dialysis CKD5 when compared to control. No difference between PD patients and controls.	Decreased in non-dialysis CKD5 Unaltered in PD
Porter et al.	1997	9434073	5	PD	*S. epidermidis*	Killing capacity by fluorescence microscopy	Decreased in non-dialysis CKD5 when compared to control as well as PD patients. No difference between PD patients and controls.	Decreased in non-dialysis CKD5 Unaltered in PD
Sardenberg et al.	2006	16155068	Non-dialysis not specified and 5	HD and PD	*S.aureus*	Analysis of uptake of labeled S.aureus by flow cytometry	Significantly lower in patients undergoing HD, but not in PD. No differences in non-dialysis CKD	Unaltered in non-dialysis CKD and PD Decreased in HD
Scalas et al.	2012	24712758	5	HD	*C.albicans*	Analysis of uptake of labeled C.albicans by flow cytometry	No significant difference	Unaltered
Schauer et al.	1991	1775265	5	HD	*S.cerevisiae*	Analysis of uptake of labeled S. cerevisiae by spectrophotometry	No significant difference	Unaltered
Talal et al.	2022	36284314	5	HD	*E.Coli* *S.aureus*	Analysis of uptake of labeled E.Coli and S.aureus by spectrophotometry	Significantly impaired	Decreased
Tullio et al.	2003	12868562	5	HD	*C.albicans*	Analysis of uptake of labeled C.albicans by flow cytometry	No significant difference	Unaltered
Ward et al.	1995	7780059	Not specified	Not specified	*S. aureus*	Analysis of uptake of labeled S.aureus by flow cytometry	No significant difference	Unaltered

[redacted]

Author	Year	PMID	CKD Stage	Dialysis type (for CKD5 patients)	Phagocytotic activity: target	Method used	Author conclusions	Increased/ decreased/ unaltered *phagocytosis* (CKD patients vs. healthy controls)
Alexiewicz et al.	1991	1951470	5	HD	*E.Coli*	Analysis of uptake of labeled E.Coli by spectrophotometry	Significantly impaired	Decreased
Alexiewicz et al.	1995	7872322	5	HD	*E.coli*	Analyis of uptake of oil droplets coated with E. coli LPS	Significantly impaired	Decreased
Anding et al.	2003	13679482	5	HD	*C.albicans*	Analysis of uptake of labeled C.albicans by flow cytometry	Slightly lower, but not statistically significant	Unaltered
Banche et al.	2006	16935897	5	HD	*K. pneumoniae*	Analysis of uptake of labeled K. pneumoniae by spectrophotometry	Compared to healthy controls, unaltered in HD when using CL-E or PS membrane but decreased when using SMC dialysis membrane	Decreased in HD when using SMC dialysis membrane Unaltered in HD when using a CL-E and PS membrane
Cuffini et al.	2001	11382696	5	HD	*K.pneumoniae*	Analysis of uptake of labeled K.pneumoniae by spectrophotometry	Lower phagocytosis after 30 and 90 min bacterial stimulation	Decreased
Lemesch et al.	2016	27698480	3 to 5	HD and PD	*E.coli*	Analysis of uptake of labeled E.coli by flow cytometry	Significantly lower in patients undergoing HD, but not in PD. No differences in non-dialysis CKD	Unaltered in non-dialysis CKD and PD Decreased in HD
Mahajan et al.	2005	16060119	2 to 5	HD	*IgG coated sheep erythrocytes*	Analysis of uptake of IgG-coated sheep erythrocytes by spectrophotometry	Compared to controls, significantly reduced in patients undergoing HD but not in non-dialysis CKD. Significantly reduced before compared to after HD session.	Unaltered in non-dialysis CKD Decreased in HD
Muniz-Junqueira et al.	2005	16005905	Non-dialysis not specified and 5	HD and PD	*S.cerevisiae*	Analysis of uptake of labeled S. cerevisiae by spectrophotometry	Significantly lower in patients undergoing HD, but not in PD. No differences in non-dialysis CKD	Unaltered in non-dialysis CKD and PD Decreased in HD
Pappas et al.	2019	31605588	5	HD	*E.Coli*	Analysis of uptake of labeled E.Coli by flow cytometry	Significantly impaired	Decreased
Patruta et al.	1998	9555668	5	HD	*E.coli*	Killing capacity by fluorescence microscopy	Lower, but not statistically significant	Unaltered
Peng et al.	2014	25339482	Not specified	Not specified	*Not specified yeasts*	Killing capacity by microscopy	Significantly impaired	Decreased
Porter et al.	1995	8569942	Non-dialysis CKD 5	PD	*S. epidermidis*	Analysis of uptake of labeled S. epidermidis by spectrophotometry	Decreased in non-dialysis CKD5 when compared to control. No difference between PD patients and controls.	Decreased in non-dialysis CKD5 Unaltered in PD
Porter et al.	1997	9434073	5	PD	*S. epidermidis*	Killing capacity by fluorescence microscopy	Decreased in non-dialysis CKD5 when compared to control as well as PD patients. No difference between PD patients and controls.	Decreased in non-dialysis CKD5 Unaltered in PD
Sardenberg et al.	2006	16155068	Non-dialysis not specified and 5	HD and PD	*S.aureus*	Analysis of uptake of labeled S.aureus by flow cytometry	Significantly lower in patients undergoing HD, but not in PD. No differences in non-dialysis CKD	Unaltered in non-dialysis CKD and PD Decreased in HD
Scalas et al.	2012	24712758	5	HD	*C.albicans*	Analysis of uptake of labeled C.albicans by flow cytometry	No significant difference	Unaltered
Schauer et al.	1991	1775265	5	HD	*S.cerevisiae*	Analysis of uptake of labeled S. cerevisiae by spectrophotometry	No significant difference	Unaltered
Talal et al.	2022	36284314	5	HD	*E.Coli* *S.aureus*	Analysis of uptake of labeled E.Coli and S.aureus by spectrophotometry	Significantly impaired	Decreased
Tullio et al.	2003	12868562	5	HD	*C.albicans*	Analysis of uptake of labeled C.albicans by flow cytometry	No significant difference	Unaltered
Ward et al.	1995	7780059	Not specified	Not specified	*S. aureus*	Analysis of uptake of labeled S.aureus by flow cytometry	No significant difference	Unaltered

Suppl. Table 5. Summary of studies on neutrophil surface markers in patients with CKD compared to healthy controls. A) Surface markers studied on neutrophils in the identified manuscripts. **B)** Neutrophils were studied in basal conditions or upon stimulation, as indicated. Studies comparing non-dialysis and dialysis CKD patients are highlighted in grey. *CKD, chronic kidney disease; fMLP, N-formyl-methionyl-leucyl-phenylalanine; HD, hemodialysis; IL-8, interleukin 8; LPS, lipopolysaccharides; PD, peritoneal dialysis; PMA, phorbol myristate acetate.*

A)

Surface marker	**Expected upon neutrophil activation**
CD11b (integrin chain)	Upregulated
CD11a (integrin chain)	Upregulated
CD18 (integrin chain)	Upregulated
CD88 (C5aR1)	Upregulated
CD66b	Upregulated
CD35 (Complement receptor type 1)	Upregulated
CD62L (L-selectin)	Downregulated (shedded)

Surface receptor	**Information**
CD15	Neutrophil marker
CXCR1	Receptor for CXCL8 (IL8)
CXCR2	Receptor for CXCL8 (IL8)
CD14	Receptor for endotoxin

[redacted]

Author	Year	PMID	CKD Stage	Dialysis type (for CKD5 patients)	Method used	Surface activation markers	Stimuli	Author conclusions	Basal Increased/decreased/unaltered *neutrophil activation* (comparing patients to healthy controls)	Stimulated Increased/decreased/unaltered *neutrophil activation* (comparing patients to healthy controls)
Aljadi et al.	2014	24712758	5	HD	Flow cytometry	CD11b, CD88	IL-8	Basal: No significant differences Stimulated with IL-8: No significant differences (Control x HD), but increased CD11b compared to unstimulated	Basal (CD11b and CD88): unaltered	Stimulated (CD11b) : unaltered
Baj et al.	2018	30135629	5	HD	Flow cytometry	CD14, TLR4, TLR9	Only basal	Basal: No significant differences for TLR4 and 9	Basal (TLR4,9): unaltered	-
Caimi et al.	2005	15665425	Not specified	Not specified	Flow cytometry	CD11a, CD11b, CD18	PMA fMLP	Basal: increased CD11b and CD18. Unaltered CD11a. Stimulated with PMA and fMLP: increased CD11b and CD18. No significant differences for CD11a	Basal (CD11b and CD18): increased Basal (CD11a): unaltered	Stimulated (CD11b and CD18): increased Stimulated (CD11a): unaltered
Cohen-Mazor et al.	2008	18319358	5	HD	Flow cytometry	CD11b	Only basal	Basal: increased	Basal (CD11b): increased	-
Dou et al.	1998	9428454	Non-dialysis not specified and 5	HD	Flow cytometry	CD62L, CD11b	Only basal	Basal non-dialysis CKD: increased CD11b, decreased CD62L Basal HD (before dialysis session): unaltered	Basal (CD11b): increased in non-dialysis CKD but unaltered in HD Basal (CD62L): decreased CD62L reflecting increased activation* in non-dialysis CKD but unaltered in HD	-
Eldewi et al.	2019	31571973	Non-dialysis 3-5	-	Flow cytometry	CD35	Only basal	Basal: decreased	Basal (CD35): decreased	-
Hassoba et al.	2007	20306662	5	HD	Flow cytometry	CD62L	Only basal	Basal: increased compared to controls, but decreased levels after dialysis session	Basal (CD62L): increased CD62L, reflecting reduced activation* After HD session: decreased CD62L, reflecting increased activation*	-
Kim et al.	2017	28523279	5	HD	Flow cytometry	CD35 and CD66b	Only basal	Basal: No significant differences (CD63 and CD66b) and increased (CD35)	Basal (CD63, CD66b): unaltered Basal (CD35): increased	-
Olsson et al.	2011	21045076	Non-dialysis 3-5	-	Flow cytometry	CD11b	LPS	Basal: increased Stimulated with LPS: significant increase in both patients with CKD and healthy controls	Basal (CD11b): increased	Stimulated (CD11b): increased
Pereira et al.	2010	20649681	5	HD	Flow cytometry	CD11b and CXCR1	Only basal	Basal: decreased CXCR1 and no significant differences for CD11b	Basal (CXCR1): decreased Basal (CD11b): unaltered	-
Talal et al.	2022	36284314	5	HD	Flow cytometry	CD18, CD11b, CD66b, CD15	Only basal	Basal: increased CD18, CD66b and CD15. No significant differences for CD11b	Basal (CD18, CD66b, CD15): increased Basal (CD11b): unaltered	-
Wallquist et al.	2018	29301137	Non-dialysis 2–5	-	Flow cytometry	CD11b and CD62L	fMLP	Basal: increased CD11b and no significant differences for CD62L	Basal (CD11b): increased Basal (CD62L*): unaltered	Stimulated (CD11b): increased Stimulated (CD62L): unaltered
Yoon et al.	2007	17136029	5	HD	Flow cytometry	CD18, CD11b	Only basal	Basal: increased	Basal (CD11b): increased Basal (CD18): increased	-
Zahran et al.	2013	24482640	Non-dialysis not specified and 5	HD	Flow cytometry	CD18	Only basal	Basal non-dialysis CKD and HD: increased	Basal (CD18): increased	-

[redacted]

Author	Year	PMID	CKD Stage	Dialysis type (for CKD5 patients)	Method used	Author conclusions	Based on PLASMA/SERUM markers: Increased/ decreased/ unaltered *degranulation* (CKD patients vs. healthy controls)	Based on ISOLATED NEUTROPHIL readouts: Increased/ decreased/ unaltered *degranulation* (CKD patients vs. healthy controls)
Capeillère-Blandin et al.	2006	16476719	2 to 5	HD	Plasma levels of MPO (by ELISA)	No significant differences in non-dialysis CKD, but significantly increased in HD patients	Unaltered in non-dialysis CKD Increased in HD	-
Costa et al.	2008	18587235	5	HD	Plasma levels of neutrophil elastase and lactoferrin by ELISA	Significantly higher elastase and unaltered lactoferrin in plasma	Elastase: increased Lactoferrin: unaltered	-
Deicher et al.	2000	10845826	5	HD	Plasma levels of lactoferrin and its release upon PMA stimulation of isolated neutrophils by ELISA	Significantly higher levels in plasma, but no significant differences in isolated neutrophils	Increased	Unaltered release (upon PMA stimulation)
Jeong et al.	2016	29130984	5	HD	Plasma levels of histone-DNA, cell-free DNA and neutrophil elastase (NE) by ELISA	Significantly higher levels of histone-DNA, but no significant differences of cell-free DNA and NE	Histone-DNA: Increased Cell-free DNA and NE: Unaltered	-
Khatib-Massalha et al.	2018	29046295	5	HD	Neutrophil elastase: Serum levels (by ELISA); activity, intracellular level (by western blotting) and surface level (by flow cytometry) in isolated neutrophils	Significantly higher serum levels, surface markers and decreased intracellular level, and activity of neutrophil elastase (more degranulation)	Increased	Reduced basal content (due to increased prior degranulation) (basal)
Otaki et al.	2004	15168383	5	HD	Release of MPO, NE and lactoferrin upon fMLP stimulation of isolated neutrophils (by ELISA)	Significantly higher MPO and NE release and reduced lactoferrin release after stimulation with fMLP	-	MPO and NE: Increased release (upon fMLP stimulation) Lactoferrin: decreased (upon fMLP stimulation)
Talal et al.	2022	36284314	5	HD	Protein levels of MPO, MMP9 and neutrophil elastase in isolated neutrophils	Significantly lower (more degranulation)	-	Reduced basal content (due to increased prior degranulation) (basal)
Tsai et al.	2013	24330098	Not specified	Not specified	Plasma levels of MPO (by ELISA)	Significantly higher	Increased	-

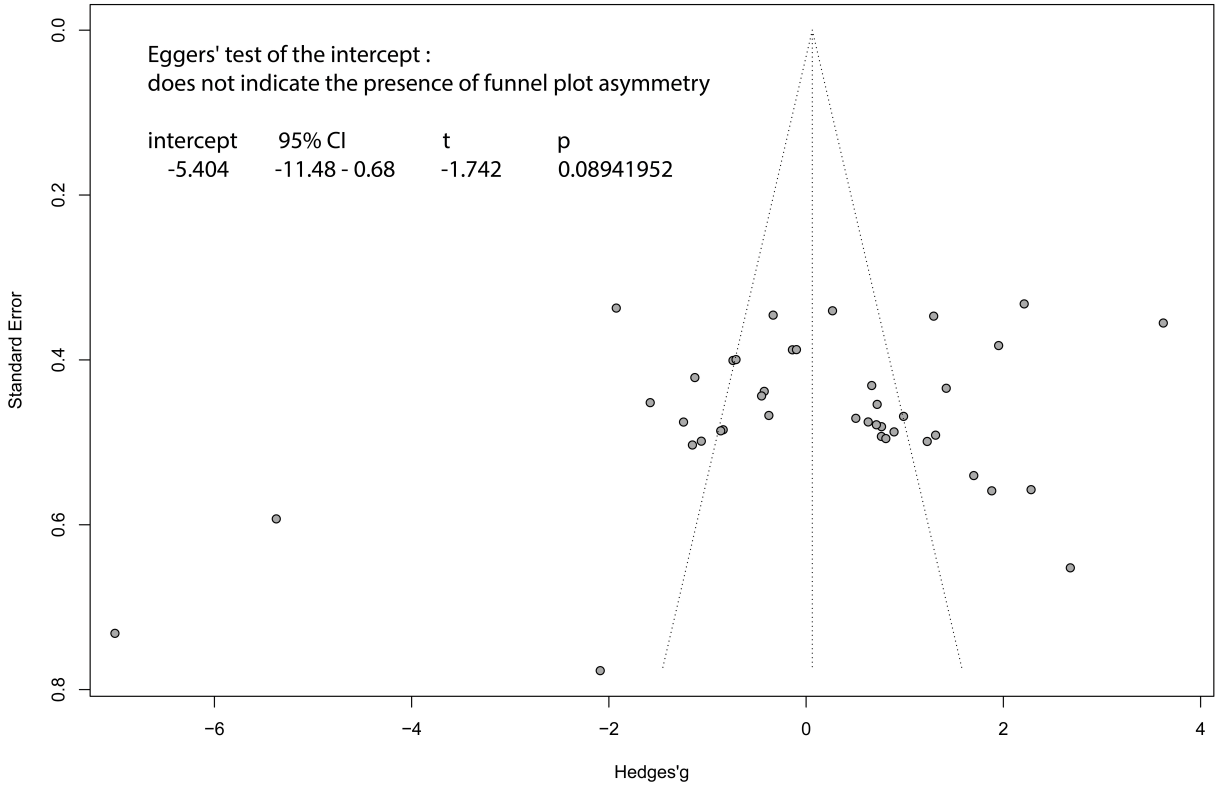
[redacted]

Author	Year	PMID	CKD Stage	Dialysis type (for CKD5 patients)	Method used	Stimuli	Author conclusions	Basal Increased/decreased/unaltered *NETosis* (comparing patients to healthy controls)	Stimulated Increased/decreased/unaltered *NETosis* (comparing patients to healthy controls)
Kim et al.	2017	1405338	5	HD	NET formation assay using Sytox green in isolated neutrophils	Basal PMA	Basal: significantly increased Stimulated with PMA: no significant differences	Increased	Unaltered (but with lower fold-increase of stimulated vs. unstimulated)
Kim et al.	2018	30296592	5	HD	Quantification of NET-releasing neutrophils via immunofluorescence of MPO and H3Cit from isolated neutrophils	Basal PMA	Significantly increased NETosis via MPO staining but not via H3Cit (low signal) Stimulated with PMA: Significantly increased NETosis via both MPO and H3Cit signal	Increased	Increased
Talal et al.	2022	36284314	5	HD	NET formation assay using Sytox green in isolated neutrophils and supernatant after PMA stimulation	Basal Calcium ionophore PMA	Basal: no significant differences Stimulated with calcium ionophore: significantly decreased DNA release compared to stimulated healthy cells, but increased compared to CKD untimulated cells. Stimulated with PMA: significantly decreased DNA release compared to stimulated healthy cells, but no differences compared to CKD untimulated cells	Unaltered	Decreased
Vega-Roman et al.	2021	2123637	5	Mixed: mainly HD/PD with few non-dialysis	NET formation assay using Sytox green in isolated neutrophils	Basal PMA	Basal: no significant differences Stimulated with PMA: no significant differences between healthy or CKD, only when comparing unstimulated with PMA-treated cells	Unaltered	Unaltered

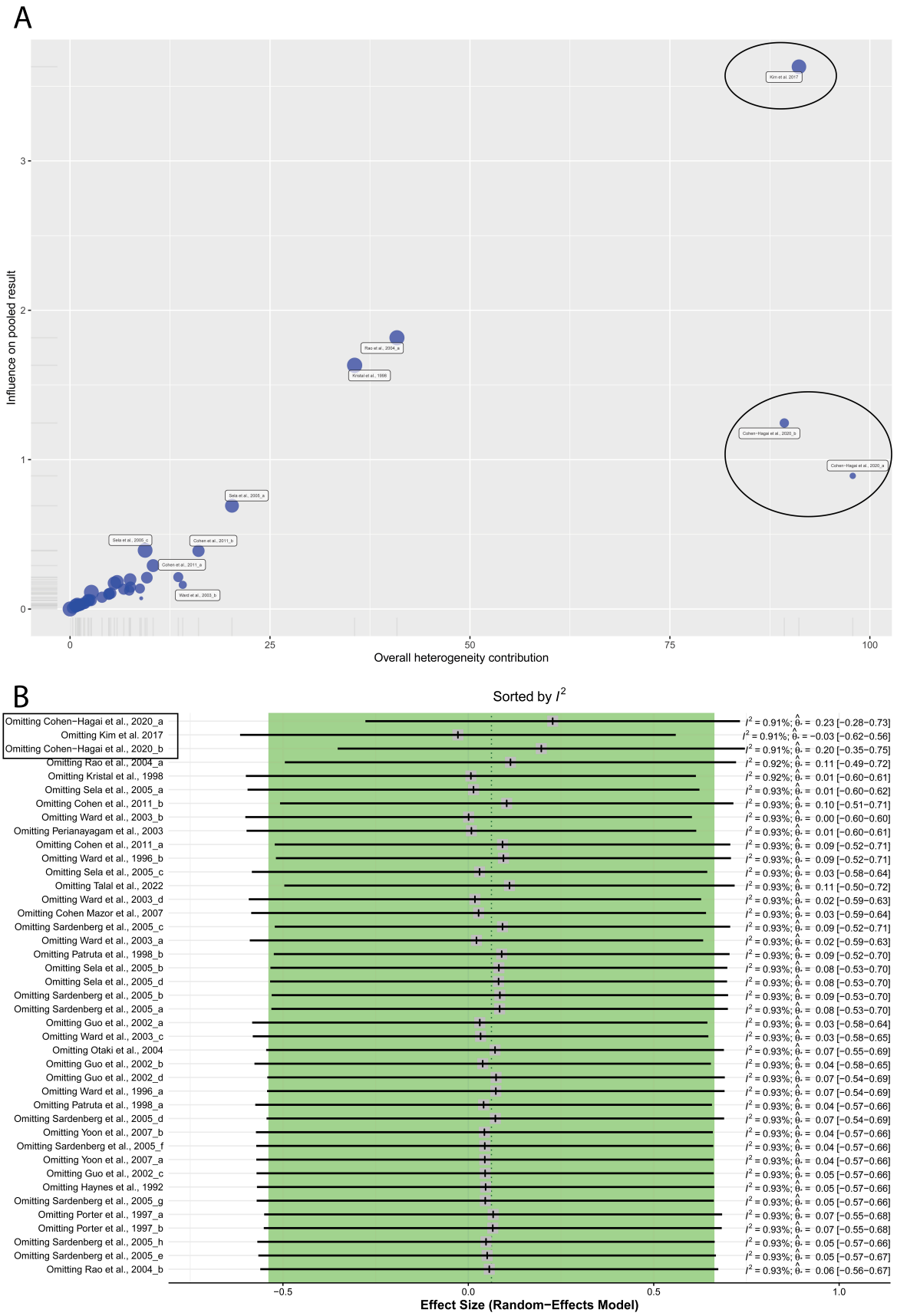
[redacted]

Study	PMID	Patient cohort		Neutrophils in full blood/isolated neutrophils	ROS Assay	Stimulus	Overall study conclusion on ROS response			
		Non-dialysis	Dialysis				Non-dialysis patients vs. healthy controls	Dialysis patients vs. healthy controls	Dialysis patients vs. non-dialysis	HD vs. PD
Anding et al., 2003	13679482	-	HD	Full blood analysis	Dihydrorhodamine	C.albicans	-	Unaltered (stimulated)	-	-
Capeillere-Blandin et al. 2006	16476719	CKD 2-4	HD	Full blood analysis	Lucigenin enhanced chemiluminescence	No stimuli	Unaltered (unstimulated)	Increased (unstimulated)	Increased (unstimulated)	-
Cohen et al., 2011	21216885	-	HD	Full blood analysis	Dihydrorhodamine	E.coli PMA	-	Decreased (stimulated)	-	-
Cohen-Hagai et al., 2020	32454385	-	HD	PMNL	Dihydrorhodamine	E.coli PMA	-	Decreased (stimulated)	-	-
Cohen-Mazor et al., 2008	18032524	-	HD	PMNL	Cytochrome c reduction	PMA	-	Increased (stimulated)	-	-
Dobos et al., 1994	8086769	-	HD	PMNL	Fluorescent para-hydroxyphenylacetic acid assay	C5a + FLPEP	-	Decreased (stimulated)	-	-
Guo et al., 2002	12227686	-	HD	PMNL	Dichlorofluorescein diacetate (DCFH-DA)	PMA fMLP S.aureus	-	Increased (unstimulated and stimulated S.aureus and fMLP) Unaltered (stimulated PMA)	-	-
Haynes et al. 1992	1328756	CKD 5	PD	PMNL	Lucigenin enhanced chemiluminescence	fMLP	Increased (stimulated)	Increased (stimulated)	Unaltered (stimulated)	-
Kierszteijn et al. 1992	1405338	-	HD	PMNL	Oxygen consumption (as readout of respiratory burst)	fMLP	-	Decreased (unstimulated and stimulated)	-	-
Kim et al. 2017	28523279	-	HD	PMNL	Dichlorofluorescein diacetate (DCFH-DA)	No stimuli	-	Increased (unstimulated)	-	-
Kristal et al., 1998	10095176	-	HD	PMNL	Cytochrome c reduction	PMA	-	Increased (stimulated)	-	-
Lemesch et al. 2016	27698480	CKD 3-5	HD and PD	PMNL	Ingestion of fluorescein-isothiocyanate (FITC) labelled opsonized bacteria	E.Coli	Unaltered (unstimulated and stimulated)	Increased (in HD, unstimulated and stimulated) Unaltered (in PD, unstimulated and stimulated)	Unaltered (stimulated)	Increased
Otaki et al., 2004	15168383	-	HD	PMNL	Dichlorofluorescein diacetate (DCFH-DA)	No stimuli	-	Unaltered (unstimulated)	-	-
Patruta et al., 1998	9555668	-	HD	PMNL	Cytochrome c reduction	PMA	-	Increased (unstimulated) Decreased (stimulated)	-	-
Perianayagam et al., 2003	14511363	-	HD	Full blood analysis	Dichlorofluorescein diacetate (DCFH-DA)	No stimuli	-	Increased (unstimulated)	-	-
Paul et al., 1991	1646409	-	HD	PMNL	Cytochrome c reduction	PMA zymosan	-	Unaltered (unstimulated) Increased (stimulated)	-	-
Porter et al., 1997	9434073	Not specified non-dialysis CKD	PD	PMNL	Dichlorofluorescein diacetate (DCFH-DA)	S.epidermidis	Decreased (stimulated)	Unaltered (stimulated)	Unaltered (stimulated)	-
Rao et al., 2004	14717942	-	HD	PMNL	Cytochrome c reduction	PMA fMLP	-	Decreased (stimulated PMA) Unaltered (stimulated fMLP)	-	-
Sardenberg et al. 2006	16155068	Not specified non-dialysis CKD	HD and PD	Full blood analysis	Dichlorofluorescein diacetate (DCFH-DA)	S.aureus fMLP PMA	Unaltered (unstimulated and stimulated)	Unaltered (in HD) Increased (in PD; S. aureus, fMLP)	Unaltered (in HD) Increased (in PD; S. aureus, fMLP, unstimulated)	Decreased (S. aureus, fMLP, unstimulated)
Sela et al. 2005	15987755	CKD 2-5	HD and PD	PMNL	Cytochrome c reduction	PMA Zymosan	Increased (stimulated PMA) Decreased (stimulated Zymosan)	Increased (stimulated PMA) Decreased (stimulated Zymosan)	Increased (in HD, stimulated PMA) Unaltered (in PD, stimulated PMA) Unaltered (stimulated Zymosan)	Unaltered
Talal et al., 2022	36284314	-	HD	Neutrophils	"ROS-ID" total ROS/ Superoxide detection kit	PMA LPS	-	Decreased (stimulated)	-	-
Ward et al. 1996	8725616	Not specified non-dialysis azotemic patients	HD	PMNL	Cytochrome c reduction	fMLP TNFα + fMLP	Unaltered (fMLP) Decreased (TNFα + fMLP)	Unaltered (fMLP) Decreased (TNFα + fMLP)	Unaltered (stimulated)	-
Ward et al. 1995	7780059	Not specified non-dialysis CKD	-	PMNL	Cytochrome c reduction	fMLP PMA	Unaltered (unstimulated) Increased (stimulated fMLP) Unaltered (stimulated PMA)	-	-	-
Ward et al.2003	12472803	-	HD	Full blood analysis	H_2O_2 measurement	S.aureus	-	Increased (unstimulated and stimulated)	-	-
Yoon et al., 2007	17136029	-	HD	Full blood analysis	Dihydroethidium (DHE), Dihydrorhodamine oxidation	No stimuli	-	Increased (unstimulated)	-	-

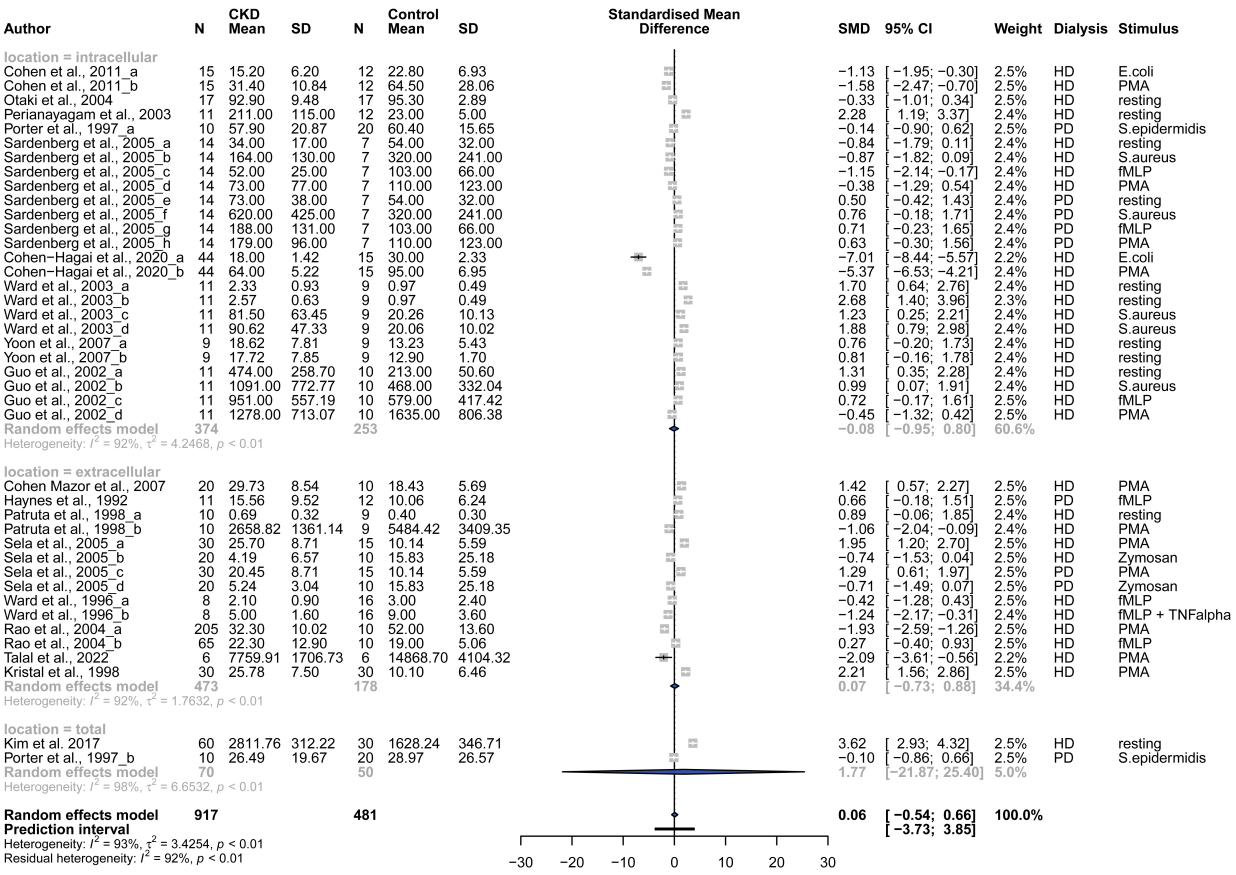
Supplementary figures



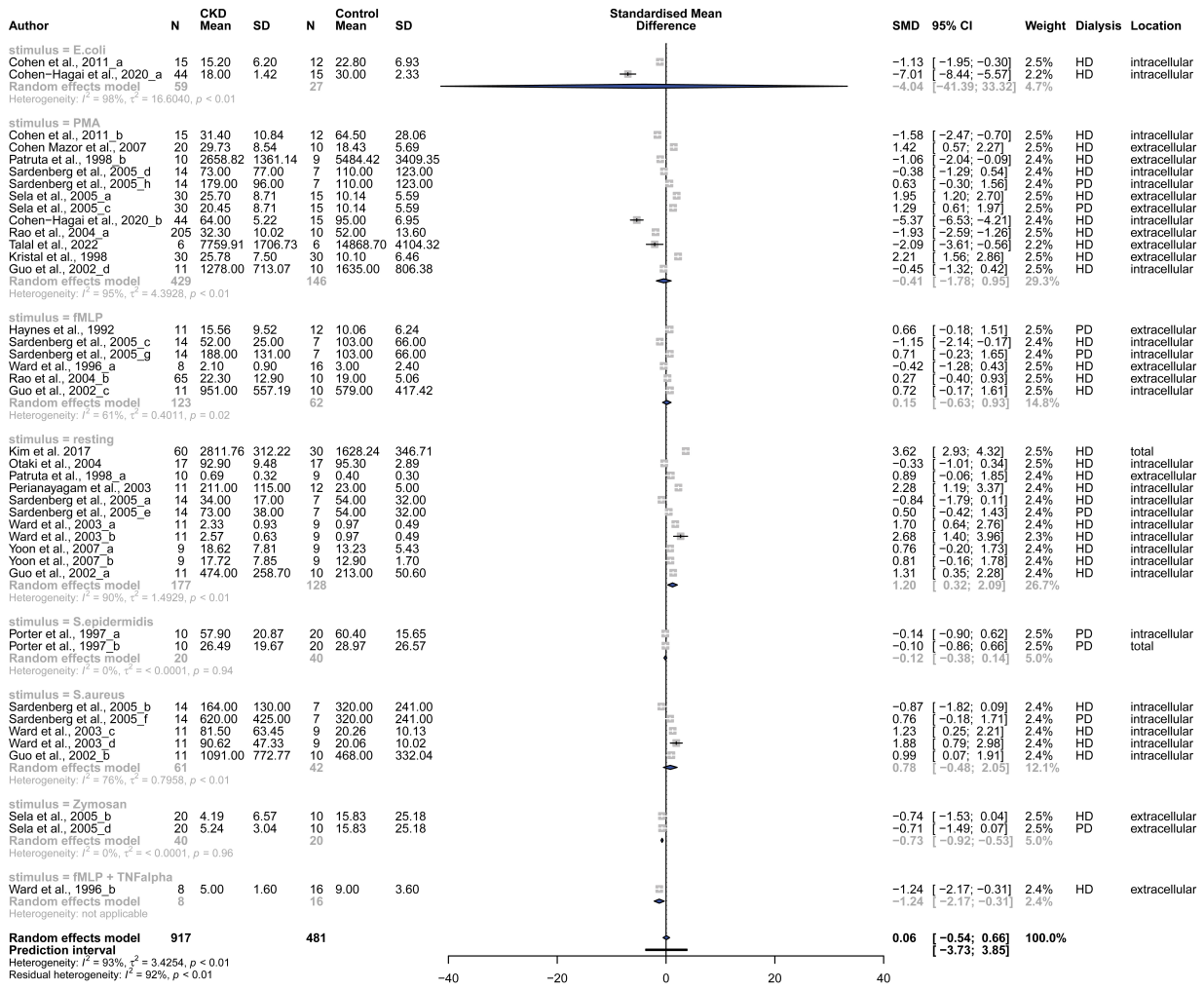
Suppl. Figure 1. Funnel plot does not indicate publication bias for studies included in the meta-analysis. Funnel plot and Eggers' test indicate the absence of publication bias.



Suppl. Figure 2. Influential outlier analysis for the studies included in the meta-analysis. Influence diagnostic plots were created for the studies included in the meta-analysis, including (A) a Baujat plot; and (B) a leave-one-out analysis sorted by I^2 value. Based on a combination of these analyses, no studies were left out for the overall meta-analysis.



Suppl. Figure 3. **Meta-analysis shows no overall significant difference in neutrophil ROS production between CKD patients on dialysis vs. controls for either intracellular or extracellular ROS.** Forest plot of ROS production in neutrophils of CKD patients receiving dialysis therapy compared to those of healthy controls, with subgroup analysis for ROS location. *CKD, chronic kidney disease; CI, confidence interval; E. coli, Escherichia coli, HD, hemodialysis; fMLP, N-formyl-methionyl-leucyl-phenylalanine; PD, peritoneal dialysis; PMA, Phorbol 12-myristate 13-acetate; S. aureus, Staphylococcus aureus; SD, standard deviation, S. epidermidis, Streptococcus epidermidis; TNFα, tumor necrosis factor α.*



Suppl. Figure 4. Meta-analysis shows increased ROS production by resting neutrophils from dialysis patients vs. controls. Forest plot of neutrophil ROS production in dialysis patients compared to healthy controls, with subgroup analysis of effect of stimulus type on ROS generation in neutrophils. The subgroup of “resting neutrophils” is displayed in zoom in Figure 4 in the main manuscript. *E. coli, Escherichia coli, HD, hemodialysis; fMLP, N-formyl-methionyl-leucyl-phenylalanine; PD, peritoneal dialysis; PMA, Phorbol 12-myristate 13-acetate; S. aureus, Staphylococcus aureus; S. epidermidis, Streptococcus epidermidis; TNFα, tumor necrosis factor α*

An dieser Stelle möchte ich mich bei allen Menschen bedanken, ohne die die Anfertigung dieser Dissertation nicht möglich gewesen wäre.

Mein besonderer Dank gilt meiner Doktormutter PD Heidi Noels, die mich über die gesamte Arbeit hinweg hervorragend betreut und unterstützt hat. Sie hat mir gezeigt, wie erstklassiges wissenschaftliches Arbeiten funktioniert.

Auch möchte ich dem gesamten IMCAR Team meinen Dank aussprechen, insbesondere Dr. Corinna Schulte, Dr. Carolina da Cruz Junho und Sonja Wohler, die mich auf meinem Weg mit Rat, Anregungen und konstruktiver Kritik unterstützt haben.

Des Weiteren möchte ich Philipp Martin, Dr. med. Susanne Fleig, Prof. Dr. med. Katharina Marx-Schütt, PD Dr. Ulrike Schulze-Späte, Prof. Dr. Oliver Söhnlein, Prof. Dr. Leticia Prates-Roma, Prof. Dr. Yvonne Döring und Dr. Constance C.F.M.J. Baaten danken. Als Co-Autoren der Publikation haben sie mir viele neue Aspekte und Blickwinkel eröffnet und mich mit ihrer Expertise unterstützt.

Außerdem möchte ich mich bei meinen Eltern bedanken, die meine Liebe zum Wissen, Lernen und der Wissenschaft von Kindesbeinen an unterstützt haben und deren guter Zuspruch und Unterstützung mich durch das gesamte Studium getragen hat.

Meinen Geschwistern und Freunden danke ich für den emotionalen Beistand, die Geduld, mit der sie sich jede Frustration anhörten und die Ablenkung, wenn ich sie brauchte.

Und zuletzt möchte ich mich bei meinem Ehemann bedanken, der bei jedem Schritt an meiner Seite war und mich immer wieder aufgebaut hat, wenn ich es am meisten brauchte.

Erklärung § 5 Abs. 1 zur Datenaufbewahrung

Hiermit erkläre ich, dass die dieser Dissertation zu Grunde liegenden Originaldaten

- Im Institut für Molekulare Herz-Kreislaufforschung (IMCAR) des Universitätsklinikums Aachen, digital in Microsoft Teams unter „Neutrophil Project“, „Manuscript Syst rev 2023“

hinterlegt sind.

Erklärung gemäß § 5 Abs. (1) und (2), und § 11 Abs. (3) 12. der Promotionsordnung

Hiermit erkläre ich, **Jane Sophie Lauxen,** an Eides statt, dass ich den wesentlichen Anteil an der Publikation:
Lauxen, J.S., Vondenhoff, S., Junho, C.V.C., Martin, P., Fleig, S., Schütt, K., Schulze-Späte, U., Soehnlein, O., Prates-Roma, L., Döring, Y., Baaten, C.C.F.M.J. and Noels, H. (2025), Neutrophil Function in Patients With Chronic Kidney Disease: A Systematic Review and Meta-Analysis. Acta Physiol, 241: e70057. https://doi.org/10.1111/apha.70057 geleistet habe.
Die Anteile an der Arbeit waren wie folgt:

Bitte Namen angeben ----------->	Jane Sophie Lauxen	Sonja Wohler, (geb. Vondenhoff)	Carolina Victoria da Cruz Junho	Philipp Martin	Susanne Fleig, Katharina Schütt, Ulrike Schulze-Späte, Oliver Soehnlein, Leticia Prates-Roma, Yvonne Döring	Constance Baaten	Heidi Noels	Summe (%)
Literaturrecherche	90						10	100
Studiendesign/Konzeption	50						50	100
Erstellung der Ein- und Ausschlusskriterien	90						10	100
Studienauswahl	90						10	100
Extraktion der Daten	80	20						100
Validierung der Daten	30	20	20	10			20	100
Datenauswertung	50	20	30					100
Statistische Auswertung	30					70		100
Visualisierung der Ergebnisse	50					30	20	100
Bereitstellung von Materialien							100	100
Interpretation der Datenauswertung	70						30	100
Verfassung des Manuskripts	**100**							100
Korrektur des Manuskripts	20	10	5	5	15	5	40	100

Aus diesem wesentlichen Anteil ergibt sich selbstverständlich die Stellung als Erstautorin.

_____
Unterschrift der Doktorandin/des Doktoranden

Als Doktormutter bestätige ich die Angaben von Jane Sophie Lauxen

_____
Unterschrift des Doktormutters