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Minireview

The role of neutrophil extracellular trap formation in kidney transplantation: Implications from donors to the recipient

Maryna van Zyl [1,2,†], Elodie Cramer [1,†], Jan-Stephan F. Sanders [3], Henri G.D. Leuvenink [4], Ton Lisman [4], Mia-Jeanne van Rooy [2], Jan-Luuk Hillebrands [1,*]

[1] Department of Pathology and Medical Biology, University Medical Center Groningen, Groningen, the Netherlands
[2] Department of Physiology, School of Medicine, University of Pretoria, Pretoria, South Africa
[3] Department of Internal Medicine, Division of Nephrology, University Medical Center Groningen, the Netherlands
[4] Department of Surgery, University Medical Center Groningen, Groningen, the Netherlands

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ABSTRACT

Kidney transplantation remains the gold standard for patients with end-stage renal disease, but severe donor organ shortage has led to long waiting lists. The utilization of expanded criteria donor kidneys within the category of deceased donors has enlarged the pool of available kidneys for transplantation; however, these grafts often have an increased risk for delayed graft function or reduced graft survival following transplantation. During brain or circulatory death, neutrophils are recruited to the vascular beds of kidneys where a proinflammatory microenvironment might prime the formation of neutrophil extracellular traps (NETs), web-like structures, containing proteolytic enzymes, DNA, and histones. NETs are known to cause tissue damage and specifically endothelial damage while activating other systems such as coagulation and complement, contributing to tissue injury and an unfavorable prognosis in various diseases. In lung transplantation and kidney transplantation studies, NETs have also been associated with primary graft dysfunction or rejection. In this review, the role that NETs might play across the different phases of transplantation, already initiated in the donor, during preservation, and in the recipient, will

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Abbreviations: ABMR, antibody-mediated rejection; ADAMTS, a disintegrin and metalloproteinase with thrombospondin motifs; AKI, acute kidney injury; C3, complement 3; C5a, complement 5a; DAMPs, danger-associated molecular patterns; eCIRP, extracellular cold-inducible RNA-binding protein; GSDMD, gasdermin D; HMP, hypothermic machine perfusion; HMGB-1, high-mobility group box 1 protein; I/R, ischemia/reperfusion; IL, interleukin; NE, neutrophil elastase; NETs, neutrophil extracellular traps; NMP, normothermic machine perfusion; PAD4, peptidyl arginine deiminase 4; ROS, reactive oxygen species; TLR, toll-like receptor; TNF-α, tumor necrosis factor-alpha; VWF, von Willebrand factor.

* Corresponding author. Department of Pathology and Medical Biology, University Medical Center Groningen, Groningen, the Netherlands.
E-mail address: j.l.hillebrands@umcg.nl (J.-L. Hillebrands).

† These authors contributed equally: Maryna van Zyl and Elodie Cramer.

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be discussed. Based on current knowledge, NETs might be a promising therapeutic target to improve graft outcomes.

1. Introduction

The burden placed on transplantation centers due to growing waiting lists and a notable donor shortage has led to the implementation of expanded criteria for donors, increasing the number of kidney transplantations that can be performed, but also increasing the risk of posttransplant complications.[1] Finding strategies that might modulate immunological activation during brain death, warm ischemia and reperfusion to reduce kidney damage is critical, not only for improved graft survival and function but also to increase the pool of transplantable kidneys.[2]

In both the donor (during brain death and ischemia) and in the recipient (during reperfusion and rejection), activation of the vascular endothelium, the production of proinflammatory cytokines and chemokines, and the simultaneous infiltration and activation of platelets and monocytes create a proinflammatory microenvironment, stimulating graft neutrophil infiltration.[3] In this proinflammatory microenvironment, activated neutrophils might be stimulated to form neutrophil extracellular traps (NETs), the release of a neutrophil's cytotoxic nuclear material to the extracellular environment. These NETs are composed of cell-free DNA and histones in web-like structures, decorated with neutrophil granular enzymes such as myeloperoxidase (MPO), neutrophil elastase (NE), and matrix metalloproteinases (MMPs).[4] NETs can be formed through either a lytic (suicidal), NADPH oxidase–dependent pathway, involving rupture of the nuclear envelope and cell membrane, or a nonlytic (vital) NADPH oxidase–independent pathway, through nuclear blebbing but with preservation of cellular function.[5] In both pathways, the neutrophil's histones are decondensed through the action of peptidyl arginine deiminase 4 (PAD4) or the proteolytic activity of MPO and NE to result in the extrusion of nuclear contents (Fig. 1A).[6]

Although NETs were first discovered as scavengers of invading pathogens,[6] the contents of NETs (histones[7] and cell-free DNA[8]) can be a source of danger-associated molecular patterns (DAMPs) during sterile inflammation, which can be recognized by pattern recognition receptors to result in the release of proinflammatory cytokines and reactive oxygen species (ROS), increased vascular permeability, and platelet activation.[9] Consequently, excessive formation and disturbance in the clearing of NETs can lead to organ damage.[10]

In various diseases, such as rheumatoid arthritis, systemic lupus erythematosus, atherosclerosis, diabetes, thrombosis, and cancer, NETs have a pathogenetic role and have been shown to be directly linked to disease severity and progression.[11]

The contribution of NETs to kidney injury has previously been explored in humans and animal models.[7,12] NETs have also been described to be correlated to increased cardiovascular events and mortality in patients with end-stage renal disease[13] and have a role in acute antibody-mediated rejection (ABMR).[14] This suggests that NETs may contribute to the pathology of graft injury and/or failure during transplantation. Although only a few studies on NET formation have been performed with a specific focus on kidney transplantation,[13-16] many studies on ischemia/reperfusion (I/R) injury may also have an application in transplantation (Table 1).[7,8,12,14-17] Furthermore, NET formation in other disease contexts is also relevant for kidney transplantation. NETs might result in suboptimal graft function through tissue damage,[18] the formation of microthrombi through activation of coagulation,[8] and enhanced activation of the recipient's immune system.[14] In this review, we will outline the potential role of NETs in kidney transplantation from the perspective of the donor, during organ preservation, and in the recipient. Furthermore, specific therapeutics that have been shown to resolve or prevent NET formation as potential intervention strategies in the context of kidney transplantation will be discussed. Currently available literature on NETs in kidney transplantation is summarized in Table 1.

2. NET formation in the donor

NETs in donor kidneys could be induced by factors known to be involved in NET formation such as DAMP's, cytokines, complement and endothelial activation, graphically depicted in Figure 1B.

2.1. DAMPs

One proposed mechanism of NET formation in the donor is through the stimulation of the neutrophil toll-like receptor 4 (TLR 4)/nuclear factor kappa-light-chain-enhancer of activated B cells pathway by DAMPs. These are molecules released from damaged cells that may result in activation of the immune system. Hypoperfusion of kidneys during both brain death and circulatory death often results in damage to the tubular epithelial cells in the donor kidney[19] which may result in the release of DAMPs like histones and high-mobility group box 1 protein (HMGB-1) or extracellular cold-inducible RNA-binding protein (eCIRP). HMGB-1 has been shown to be a potent inducer of NETs either through neutrophil stimulation with platelet-expressed HMGB-1,[20] or through soluble HMGB-1 released from damaged cells.[21] Histones might in turn prime neutrophils for NET formation, predisposing neutrophils for NET formation through other stimuli, as demonstrated through in-vitro studies.[7] eCIRP, an RNA chaperone, has also recently been identified as a DAMP and was shown to be involved in inflammation during hemorrhagic shock and sepsis,[22] which might suggest relevance to brain or circulatory death because these conditions are also associated with a systemic inflammatory response. eCIRP has been demonstrated to directly induce NET formation in in-vitro studies[23]; thus, a potential increase in eCIRP in donors might mediate increased NET formation.

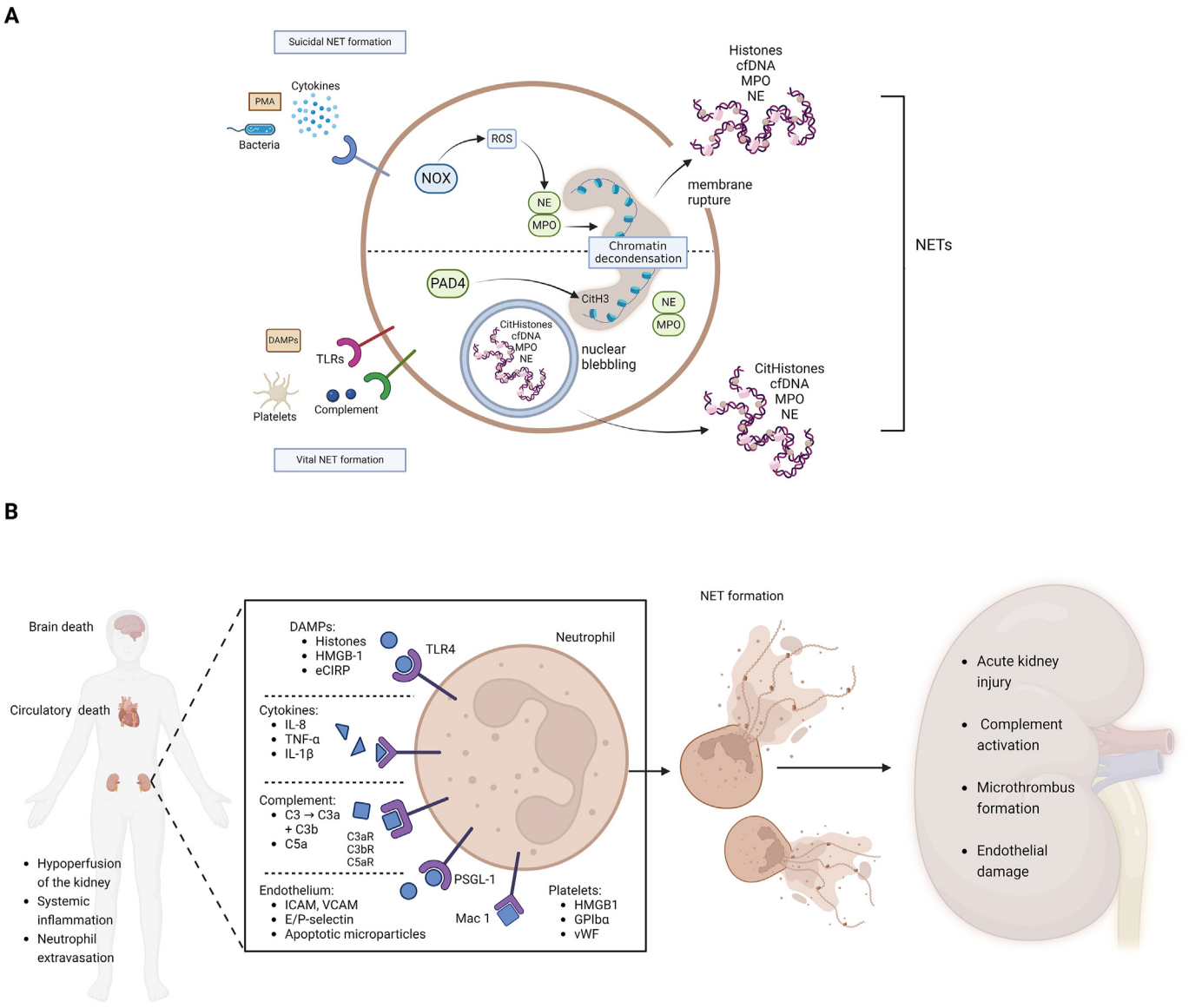

Figure 1. Pathways of neutrophil extracellular trap (NET) formation and NETs in the donor. (A) NETs may either be formed through the suicidal or vital pathway, in response to cytokines, microbes, platelets, and danger-associated molecular patterns (DAMPs). In both pathways, histones are decondensed to result in the release of the nuclear contents to the extracellular environment. (B) NET formation in the deceased donor can be mediated via neutrophil stimulation with DAMPs, cytokines, complement, activated endothelial cells, and platelets. NETs themselves may contribute to acute kidney injury, enhance complement activation, contribute to microthrombus formation, and cause endothelial damage. Figure created with Biorender.com. C3, complement 3; C5a, complement 5a; cfDNA, cell-free DNA; CitH3, citrullinated histone 3; eCIRP, extracellular cold-inducible RNA-binding protein; GP, glycoprotein; HMGB1, high-mobility group box 1; ICAM, intercellular adhesion molecule; IL, interleukin; Mac, macrophage antigen; MPO, myeloperoxidase; NE, neutrophil elastase; NOX, NADPH oxidase; PMA, phorbol 12-myristate 13-acetate; PAD4, peptidyl arginine deiminase; PF, platelet factor; PSGL, P-selectin glycoprotein 1; ROS, reactive oxygen species; TLR, toll-like receptor; TNF-α, tumor necrosis factor-alpha; VCAM, vascular cell adhesion molecule; VWF, von Willebrand factor.

2.2. Inflammatory cytokines

The massive amount of catecholamines released during brain death leads to increased inflammation and the consequent release of pro-inflammatory cytokines such as interleukin (IL)-8, interferon-gamma, tumor necrosis factor-alpha (TNF-α), IL-6, and IL-1 beta (β).[24] Similarly, the period of warm ischemia during circulatory death primes the activation of immune cells and cell death pathways which also culminates in the release of chemokines and cytokines.[25] The surge in inflammatory cytokines in the systemic circulation may either prime neutrophils for increased NET formation through other mediators or directly induce NET formation, especially through TNF-α, IL-1β, and IL-8.[26]

2.3. Complement activation

In both brain-dead and circulatory-dead donors, complement activation has been shown both locally and systemically. Increased expression of the acute phase protein, complement 3 (C3),[27] elevated circulating levels of complement 5a (C5a), and

Table 1
Current available literature on the potential role of NETs in kidneys.

Article	Findings	Application to transplantation	Type of study	Reference
A potential role of neutrophil extracellular traps (NETs) in kidney acute antibody-mediated rejection	Neutrophils from transplanted patients and patients with rejection form spontaneous NETs. NETs are present in biopsies of patients with ABMR.	Recipient/posttransplant complication	Human/in-vitro	Torres-Ruiz et al[14]
Circulating ‘‘Neutrophil extracellular traps’’ during the early postrenal transplant period and correlation with graft dysfunction and rejection	Regardless of graft outcome, circulating NETs increase in kidney transplant recipients in the posttransplant period.	Recipient/posttransplant complication	Human	Kumar et al[15]
Identification of renal ischemia reperfusion injury subtypes and predictive strategies for delayed graft function and graft survival based on neutrophil extracellular trap-related genes	Based on differentially expressed NET-related genes, 2 I/R clusters could predict delayed graft function and graft survival.	Recipient	Human	Wu et al[16]
Histones and neutrophil extracellular traps enhance tubular necrosis and remote organ injury in ischemic AKI	Renal ischemia induces the release of DAMPs from tubular cells, stimulating NET formation in in-vitro and animal models.	Donor/recipient	In-vitro/animal/human	Nakazawa et al[7]
Release of extracellular DNA influences renal ischemia reperfusion injury by platelet activation and formation of neutrophil extracellular traps	I/R injury leads to platelet-mediated NET formation in mouse kidneys. Platelet inhibition, reduced NET formation.	Recipient	In-vitro/animal	Jansen et al[8]
Neutrophil peptidyl arginine deiminase-4 has a pivotal role in ischemia/reperfusion-induced acute kidney injury	PAD4 in kidney infiltrated leukocytes are upregulated following I/R, resulting in the formation of NETs. Mice with PAD4 deficiency did not form NETs.	Recipient/preservation	In-vitro/animal	Raup-Konsavage et al[17]
Reduced neutrophil extracellular trap formation during ischemia reperfusion injury in C3 KO mice: C3 requirement for NETs release	I/R injury induces the formation of NETs and C3 expression. Mice with C3 deficiency did not produce NETs.	Recipient	In-vitro/animal	Wu et al[12]

ABMR, antibody-mediated rejection; AKI, acute kidney injury; C3, complement 3; DAMPs; danger-associated molecular patterns; I/R; ischemia/reperfusion; KO, knockout; NET, neutrophil extracellular trap; PAD4, peptidyl arginine deiminase.

increased C5aR expression in the renal tubules have been observed in brain-dead donors,[28] with a similar trend observed in circulatory-dead donors.[29] The relationship between NET formation and complement has already been thoroughly reviewed elsewhere.[30] In short, C3a may induce NET formation by stimulating the C3a receptors on neutrophils. This has been demonstrated by the inability of neutrophils to produce NETs following C3[31] and C3a-receptor (R) knockout.[32] In addition, C5a has been highlighted as both a potent secondary inducer of NET formation when neutrophils are pretreated with interferon-gamma[33] or a primary inducer of NET formation.[34] The interaction of C5a with C5aR on neutrophils is also known to activate neutrophils, increasing chemotaxis and the generation of ROS.[28] In-vitro experiments revealed an increase in mitochondrial ROS following neutrophil incubation with C5a, and subsequent NET formation could be reduced through the inhibition of mitochondrial ROS production.[35]

2.4. Endothelial dysfunction

Traumatic brain injury leading to brain death is associated with vascular endothelial activation as a result of systemic changes such as hemodynamic instability, a cascade of inflammatory cytokines, and hormonal changes, culminating in impaired nitric oxide synthesis, production of ROS, increased expression of adhesion molecules (intercellular adhesion molecule-1, vascular adhesion molecule-1), E-selectin, and proteins that participate in hemostasis (von Willebrand factor [VWF]).[36] In a similar fashion, prolonged warm ischemia during circulatory death may also enhance the expression of adhesion molecules or TLRs on endothelial cells.[37] An increase in proinflammatory cytokines and adhesion molecule expression generates a chemotactic gradient, increasing the infiltration of immune cells and platelets into the kidney.[36] It has previously been shown that activated endothelial cells can induce NET formation, potentially through neutrophil interaction with adhesion molecules on endothelial cells or inflammatory cytokines produced by endothelial cells.[38] Reciprocally, NETs can also impact donor graft endothelium by causing increased endothelial cell death and vascular permeability.[38]

2.5. Platelet activation

Activation of platelets during brain and circulatory death has been well established.[39] The granular contents released by activated platelets add to the pool of proinflammatory cytokines and cytotoxic molecules, enhancing the proinflammatory microenvironment.[40]

Brain death has also been associated with an imbalanced VWF/a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS13) axis which mediates platelet-platelet, platelet-neutrophil as well as platelet-endothelium interactions. Low circulating levels of ADAMTS13, an enzyme functioning in the cleavage of ultralarge VWF to smaller fragments, have been reported in brain-dead donors.[41] An imbalanced VWF/ADAMTS13 ratio can lead to microthrombi formation in the donor organs as a result of insufficient cleavage of ultralarge, highly thrombotic VWF, and subsequent increased platelet-neutrophil interaction.[42]

Platelets have a prominent role in the formation of NETs. Various mechanisms of action have been proposed, including direct interaction of macrophage antigen 1 (Mac-1) on neutrophils with the glycoprotein Ibα on platelets,[43] and the interaction of platelet P-selectin with P-selectin glycoprotein ligand-1 on neutrophils.[44] Platelets also mediate NET formation through the expression of HMGB-1 and presentation to TLRs on neutrophils. In-vitro analyses have shown that HMGB-1-deficient platelets do not support NET formation.[20] Lastly, platelet-derived VWF and platelet factor 4 have also been found to play a critical role in NET formation through a thromboxane A_2–dependent pathway.[45]

3. Organ preservation

The extent of I/R injury can be reduced through some preservation techniques; thus, the impact of different preservation techniques is also relevant for NET formation because reduced I/R injury might also limit NET formation. Currently, hypothermic storage may either be static, known as static cold storage, or dynamic, through hypothermic machine perfusion (HMP).[46] During HMP, many cells, including neutrophils, are flushed out of the renal circulation, which might reduce the number of cells able to form NETs within the kidney. Hypothermic oxygenated perfusion might also reduce NET formation following reperfusion by reducing ischemic injury during preservation.[47]

Normothermic machine perfusion (NMP), which mimics near-physiological conditions characterized by optimal oxygen delivery and stimulation of normal metabolic function, might also have an impact on NET formation. Optimal oxygen delivery through perfusion with a red blood cell–based solution could minimize ischemic injury to the kidney when transplanted, which could thereby also reduce NET formation.[48,49] It has however also been found that NMP activates complement and increases the release of proinflammatory cytokines (IL-6, IL-8, and TNF).[50] NMP may therefore also potentially enhance NET formation, as complement and cytokine production are reciprocally linked to NET formation.[30]

Preservation techniques should thus be carefully evaluated (static cold storage vs HMP vs NMP), because different factors involved such as the dynamic vs static nature of preservation, oxygenation, and temperature may potentially reduce or enhance NET formation, causing graft injury (Fig. 2).

4. The recipient

Upon implantation, in addition to residual NETs from the donor, NETs may also be derived from the recipient's neutrophils. Neutrophil activation and subsequent NET formation in the recipient may occur because of I/R injury or due to increased immune activation as a result of host immune responses. In a single-center prospective cohort study, Vega-Roman et al[13] showed that elevated circulating markers of NETs in patients with end-stage renal disease were not reduced after transplantation. Interestingly, Kumar et al[15] reported that circulating NETs are elevated in the early posttransplant period, regardless of graft

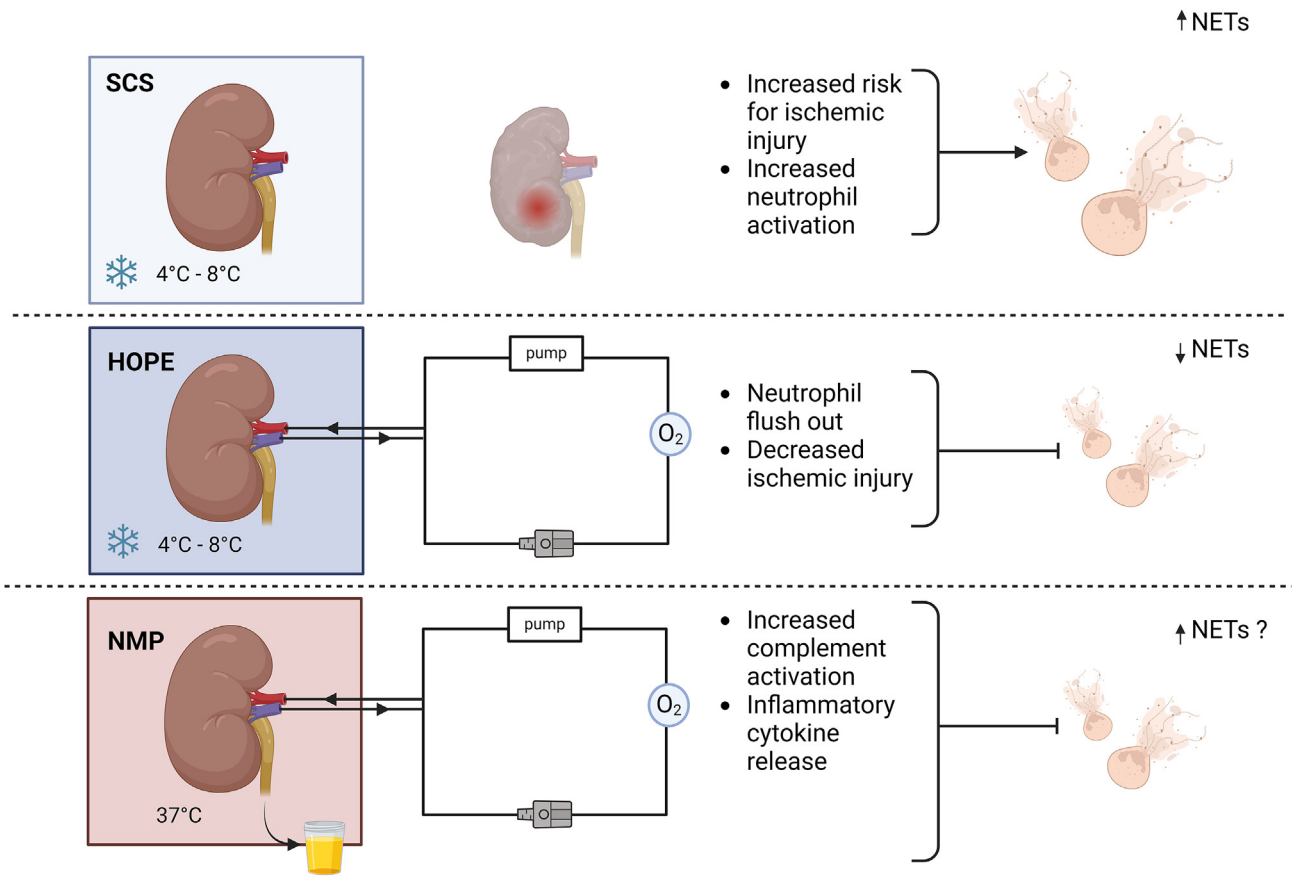

Figure 2. The impact of different preservation techniques on neutrophil extracellular trap (NET) formation. During preservation, the formation of NETs might depend on the type of preservation utilized. It is suspected that static cold storage (SCS) might result in an increase in residual NETs due to inadequate flush-out and increased NET formation following reperfusion due to greater ischemic injury. Figure created with biorender.com. HOPE, hypothermic oxygenated perfusion; NMP, normothermic machine perfusion.

outcome. Although the study did not find a significant correlation between malondialdehyde, a marker of I/R injury, and NETs, a potential link between I/R and circulating NETs in recipients might still be plausible, possibly measured in a larger cohort or through additional injury markers. In contrast, Wu et al[16] reported an overlap between I/R and NET-related genes, which were also predictive for delayed graft function.

4.1. I/R injury

Ischemia in the donor or during preservation primes the graft for injury during reperfusion through a buildup of calcium and calcium-dependent proteolytic enzymes, and the generation of ROS, causing changes in endothelial integrity, a decrease in nitric oxide synthesis and an increase in expression of adhesion molecules. During reperfusion, the excessive production of ROS and calcium overload triggers the opening of the mitochondrial permeability transition pore, stimulating several cell death pathways such as apoptosis and necrosis, while activating the innate and adaptive immune system.[25] It was shown that kidneys exposed to long periods of cold ischemia had increased NET formation following reperfusion, compared to grafts with shorter cold ischemia periods. The presence of NETs and tubular injury were also positively correlated in a time-dependent manner.[7] The inhibition of NETs also reduced the severity of ischemic acute kidney injury (AKI).[7,17] Results from these studies suggest an association between the duration of ischemia, NET formation, and injury following reperfusion. AKI, which already started in the donor as injury or cell death to tubular epithelium and endothelium, may result in increased infiltration of recipient neutrophils, while the continuous release of DAMPS, (HMGB-1, histones and DNA) by damaged cells, stimulates infiltrated neutrophils to produce NETs, exacerbating tissue injury.[7]

PAD4, an enzyme involved in NET formation, has also been shown to have an important role during NET-mediated I/R injury. PAD4 knockout mice did not have NET formation and no significant kidney injury, wild-type mice had an increase in PAD4 levels and reduced kidney function after I/R, with a substantial loss of tubular epithelial cells, upregulation of TNF-α, IL-6, and IL-8, and enhanced NET formation.[17] Accordingly, inhibition of PAD4 concomitantly also reduces circulating NETs and tubular necrosis in an I/R injury model.[7] Other contributors to NET formation in the recipient during I/R injury may include mediators of coagulation and complement, similar to the donor setting. Jansen et al[8] observed that the DNA released from necrotic tubular epithelial cells can activate platelets, thereby enhancing NET formation. This was illustrated through the colocalization of platelets with NETs in a mouse model, and NET release following tubular

epithelial cell, platelet, and neutrophil coculture experiments. Mice that were treated with clopidogrel, a platelet function inhibitor, had reduced NET formation, suggesting that platelets play an important role in NET formation during I/R injury.[8] This is in line with a previously reported decrease in risk for I/R-mediated renal failure in patients treated with aspirin prior to surgery.[51]

During I/R injury, the concurrent expression of C3, neutrophil infiltration, and NET formation were also observed. C3 knockout mice presented with decreased I/R injury, infiltrated neutrophils, and NET formation, suggesting C3-mediated NET formation during I/R.[12]

An intersection between gasdermin D (GSDMD), a pore-forming molecule and downstream mediator of pyroptosis, and NET formation might also be relevant during I/R injury. Various studies have described the role of GSDMD in NE activation and plasma membrane perforation during NET formation. In addition to caspase-11, GSDM can also be activated through NE, forming a feedforward loop of NET generation.[52,53] The inhibition of GSDMD in lungs subjected to I/R, decreased NET release and injury,[54] and reduced organ dysfunction in a sepsis model.[55] These results could be extrapolated to I/R injury following kidney transplantation because I/R injured kidneys have been shown to abundantly express GSDMD and caspase-11.[56] The role of GSDMD-dependent NET formation in renal transplantation should be explored in future studies as caspase 11/GSDMD–mediated NET formation has been demonstrated to contribute to renal fibrosis through inflammation and macrophage-to-myofibroblast transition.[52]

The impact of I/R injury on potential NET formation in the recipient is summarized in Figure 3.

4.2. NETs in human posttransplant complications

In various types of organ transplants, NET formation has been associated with rejection or graft dysfunction. Torres-Ruiz et al[14] established the presence of NETs in both the kidney biopsies

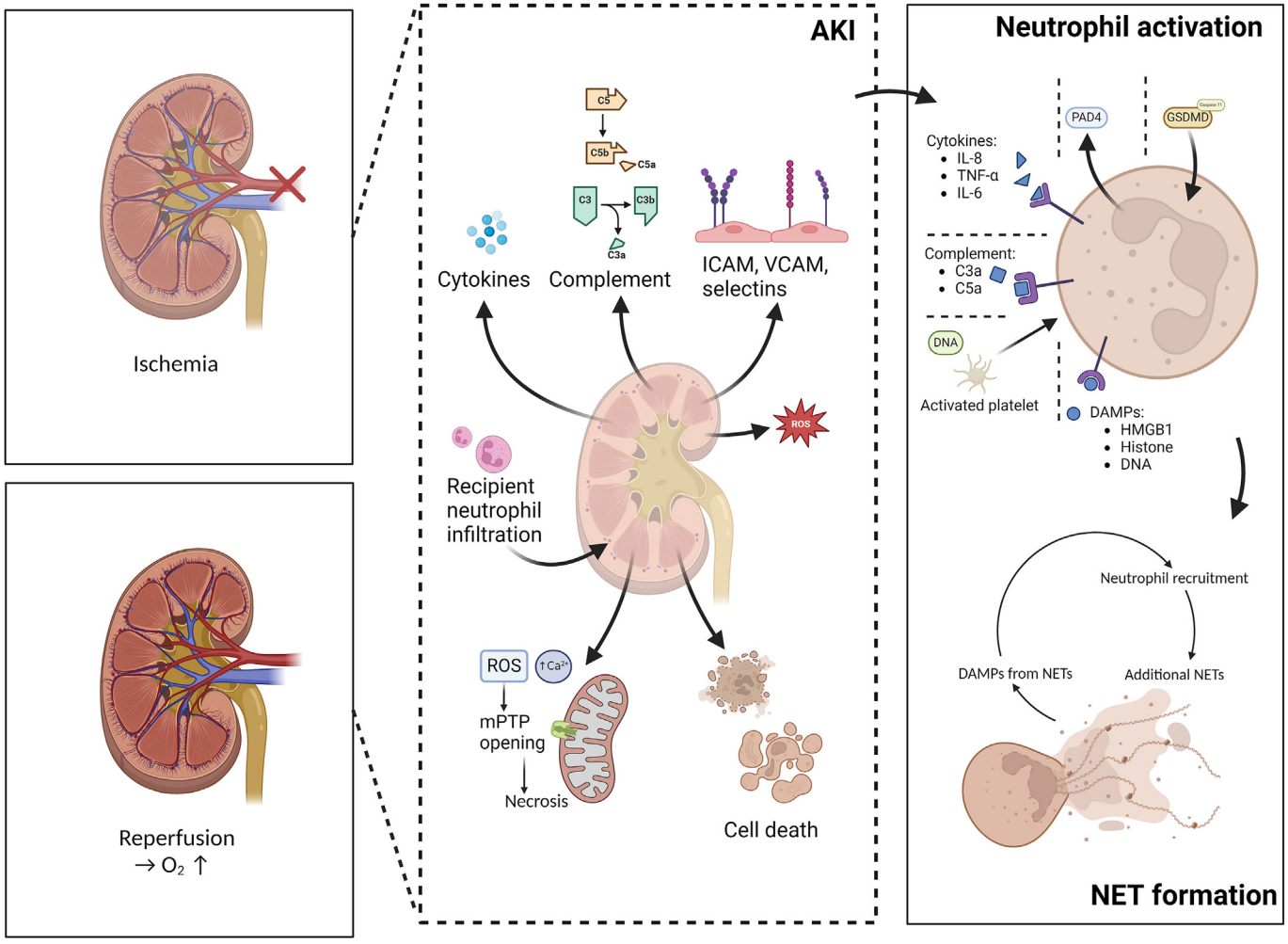

Figure 3. Neutrophil extracellular traps (NET) formation in the recipient. Following ischemia/reperfusion, the activation of complement, cytokine release, increased cellular infiltration, production of ROS, the opening of the mitochondrial permeability transition pore (mPTP), and cell death results in acute kidney injury (AKI). Infiltrated neutrophils can be activated by cytokines, cell-free DNA (cfDNA), platelets, danger-associated molecular patterns (DAMPs), and caspase 11/GSDMD to release NETs, which results in the recruitment of more neutrophils, feeding back into more NET formation and enhancement of kidney tissue injury. Figure created with biorender.com. C3, complement 3; C5a, complement 5a; Ca^{2+}, calcium; GSDMD, gasdermin D; HMGB, high-mobility box; ICAM, intercellular adhesion molecule; IL, Interleukin; O_2, oxygen; PAD, peptidyl arginine deiminase; ROS, reactive oxygen species; TNF-α, tumor necrosis factor-alpha; VCAM, vascular cell adhesion molecule.

and in the circulation of patients with ABMR. Isolated neutrophils of patients with ABMR also had an increased capacity for NET formation upon stimulation and spontaneously formed NETs. In liver transplant recipients, an independent relationship between NETs and acute rejection has been demonstrated.[57] The formation of NETs has also been linked to primary graft dysfunction in the lungs. Sayah et al[58] showed that NET-mediated primary graft dysfunction may be facilitated by platelets. The presence of NETs in the circulation and in lung biopsies of patients with rejection in contrast to low levels in patients without rejection, was also confirmed by Bonneau et al.[18] Levels of circulating NETs were positively correlated with the progression of primary graft dysfunction, implicating a pathogenetic role of NETs in primary graft dysfunction. The patients with severe primary graft dysfunction also presented with platelet depletion, potentially indicating systemic NET-associated thrombus formation.[18] These results could also be relevant for kidney transplant patients because rapid platelet infiltration and concomitant complement activation have been demonstrated in a mouse model during the initial stages of humoral kidney rejection.[59]

Specific interactions between NET formation and complement activation may also be relevant during posttransplant complications. The complement system can be activated by donor-specific antibodies, which is associated with a 3 times higher risk for allograft loss compared to instances in which donor-specific antibodies are present without complement activation.[60] A study assessing C5b-9 serum levels 10 weeks posttransplant found that increased C5b-9 levels correlated with reduced long-term graft and patient survival.[61] C3 is also associated with decreased graft function after transplantation, whereas inhibition of C3 prolonged graft survival and reduced incidence of ABMR in a primate model.[27] The direct relationship between these complement factors and NET formation has already been explored; thus, in this context, complement might not only directly contribute to rejection but also indirectly through stimulation of increased NET formation.

The role of NETs in xenograft rejection has also received increasing attention due to the unique neutrophil, macrophage- and natural killer cell–mediated dominant reactions in xenotransplantation, compared to allogeneic rejection which is mainly mediated by T and B cells.[62] By using a 3D vessel chip, Yadav et al[63] demonstrated NET formation in human neutrophils during coculture experiments with porcine aortic endothelial cells. The phenomenon of neutrophil-driven cellular rejection through NET formation was further investigated by Wang et al,[64] who reported decreased NET-mediated cytotoxicity following human neutrophil exposure to swine endothelial cells when swine cells were transfected to express human CD31. Compared to human endothelium, porcine endothelium results in increased neutrophil activation, chemotaxis, and adherence through porcine-specific endothelium secretions.[65] In kidney xenotransplantation, targeted therapies toward both neutrophils and the endothelium, might therefore mitigate NET formation to potentially attenuate xenograft rejection.

5. Intervention

Although many pharmacological interventions have been proven successful at inhibiting or resolving NET formation in animal and in-vitro models (Table 2),[17,20,66-70] in humans, a clinically applicable method to prevent and resolve NETs does not yet exist in the context of kidney transplantation. Some NET interventions with potential in the context of transplantation include the following: digestion of the DNA backbone located in NETs through DNAse[17] (which is currently already Food and Drug Administration–approved for use in the clinic in certain diseases such as cystic fibrosis), PAD4 inhibitors (Cl-amidine, GSK199, and GSK484),[66] antiplatelet agents (aspirin or P2Y12 blockers such as clopidogrel and ticagrelor),[8] inhibition of platelet-derived HMGB-1 with BoxA,[20] inhibition of complement (eculizumab, blocking the cleavage of C5 and assembly of MAC)[67] or immunosuppressants (cyclosporine A[68] and rapamycin[69]) (Table 2). Alternatively, novel therapeutics that may remove NETs from the circulation or perfusate such as the NucleoCapture selective apheresis device, which has already been tested in animal lung transplantation models,[70] could also be considered during kidney transplantation. Different treatment strategies against NET formation are summarized in Table 2.

During treatment, the different phases of transplantation could potentially also be utilized for intervention against NET formation. Because treatment in the donor might not yet be possible due to logistics and ethical concerns, treatment of kidneys during preservation (machine perfusion) might be a promising alternative to eliminate preformed donor-derived NETs before transplantation.[71] Treatment on the pump may also reduce the risk of drug side effects in the recipient and lower dosage costs.[72] Ex-vivo perfusion, however, would only remove donor-derived

Table 2
Potential treatment strategies for the resolution of NETs in kidney transplantation.

Treatment	Mechanism	Reference
DNAse	Breakdown of NETs	Raup-Konsavage et al[17]
GSK 199, GSK 484, Cl-amidine	PAD4 inhibitors	Lewis et al[66]
BoxA	HMGB-1 inhibition	Maugeri et al[20]
Eculizumab	Complement inhibition	Skendros et al[67]
Cyclosporine A	Calcineurin inhibitor	Gupta et al[68]
Rapamycin	mTOR inhibitor	McInturff et al [69]
NucleoCapture selective apheresis device	Removal of circulating NETs	Mittendorfer et al[70]

HMGB1, high-mobility group box 1 protein; mTOR, mammalian target of rapamycin; NETs, neutrophil extracellular traps; PAD4, peptidyl arginine deiminase.

NETs and does not account for de novo NET formation following reperfusion or during rejection, although grafts with reduced donor NETs would likely also result in reduced I/R injury and reduced recipient NET formation.

6. Conclusion

NETs may be formed in different phases of transplantation to potentially contribute to inferior graft quality and outcome through multiple mediators such as DAMPs, proinflammatory cytokines, interactions with platelets and endothelium, and the complement system. It has already been established that NETs may worsen I/R injury and actively cause AKI, and could therefore independently contribute to graft injury. To date, few studies have been conducted on NETs in patient cohorts in the specific setting of kidney transplantation. This provides a promising avenue for research to not only investigate the presence of NETs in the donor and transplanted graft but also elucidate the underlying mechanisms of NET formation in this setting. Tissue biopsy and circulating NETs have been associated with ABMR,[14] and NET-related genes could predict delayed graft function[16] in kidney recipients. In addition, circulating NETs were associated with the progression of primary graft dysfunction in lung transplant recipients.[18] NETs as a biomarker of graft outcome in kidney transplantation might therefore be promising, but needs further investigation, not only in the recipient but also in the donor. Currently, various established and novel therapies exist against NET formation, which need to be tested in the kidney transplant setting. The formation of NETs across different phases of transplantation should be analyzed, as this may suggest when intervention would be optimal.

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Author contributions

E.C. and M.vZ. drafted the manuscript; J.S.F.S., H.G.D.L., T.L., M.J.vR and J.L.H. edited and reviewed the manuscript.

Data availability

Data availability and sharing are not applicable to the article because no new data was generated or analyzed for this minireview.

Declaration of competing interest

The authors of this manuscript have no conflicts of interest to disclose as described by the American Journal of Transplantation.

ORCiD

Maryna van Zyl https://orcid.org/0009-0005-6227-1740
Elodie Cramer https://orcid.org/0009-0000-9145-3635
Jan-Stephan F. Sanders https://orcid.org/0000-0002-0904-3969
Henri G.D. Leuvenink https://orcid.org/0000-0001-5036-2999
Ton Lisman https://orcid.org/0000-0002-3503-7140
Mia-Jeanne van Rooy https://orcid.org/0000-0003-1224-5147
Jan-Luuk Hillebrands https://orcid.org/0000-0003-3135-3274

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