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Mitochondrial Damage-associated Molecular Patterns as Potential Biomarkers in DCD Heart Transplantation: Lessons From Myocardial Infarction and Cardiac Arrest

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Abstract. Heart transplantation with donation after circulatory death (DCD) has become a real option to increase graft availability. However, given that DCD organs are exposed to the potentially damaging conditions of warm ischemia before procurement, new strategies for graft evaluation are of particular value for the safe expansion of DCD heart transplantation. Mitochondria-related parameters are very attractive as biomarkers because of their intimate association with cardiac ischemia-reperfusion injury. In this context, a group of mitochondrial components, called mitochondrial damage-associated molecular patterns (mtDAMPs), released by stressed cells, holds great promise. mtDAMPs may be released at different stages of DCD cardiac donation and may act as indicators of graft quality. Because of the lack of information available for DCD grafts, we consider that relevant information can be obtained from other acute cardiac ischemic conditions. Thus, we conducted a systematic review of original research articles in which mtDAMP levels were assessed in the circulation of patients with acute myocardial infarction and cardiac arrest. We conclude that 4 mtDAMPs, ATP, cytochrome c, mitochondrial DNA, and succinate, are rapidly released into the circulation after the onset of ischemia, and their concentrations increase with reperfusion. Importantly, circulating levels of mtDAMPs correlate with cardiac damage and may be used as prognostic markers for patient survival in these conditions. Taken together, these findings support the concept that mtDAMPs may be of use as biomarkers to assess the transplant suitability of procured DCD hearts, and ultimately aid in facilitating the safe, widespread adoption of DCD heart transplantation.

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INTRODUCTION

For patients suffering from advanced stages of heart failure, heart transplantation remains the gold standard therapy to improve quality of life and survival. However, the number of grafts is insufficient to cover the ever-increasing demand for transplants. This has driven the search for alternative sources of cardiac grafts for transplantation.

One promising approach to reduce the graft shortage is the use of hearts obtained from donation after circulatory death (DCD), with >140 transplantations performed since 2014.[1] Two main clinical protocols for DCD heart procurement are routinely used: (i) direct procurement and perfusion, in which DCD hearts are procured immediately after declaration of death and delivery of cardioplegia, and (ii) normothermic

regional perfusion, in which DCD hearts are returned to a beating state in the donor after exclusion of cerebral circulation. In both protocols, grafts are transported between centers using normothermic, ex-situ heart perfusion (ESHP) with the Transmedics Organ Care System.[2] These clinical protocols have provided short- and mid-term outcomes for recipient survival and rejection episodes comparable to matched transplantation cohorts of conventional donation after brain death (DBD).[3,4]

Despite the positive results obtained with DCD heart transplantation, concerns persist regarding its adoption.[5] DCD hearts are exposed to variable conditions in the donor, including increased levels of circulating catecholamines, periods of hemodynamic instability and warm ischemia, which may incur rapid and severe cardiac damage. These damaging conditions are of particular concern as graft evaluation often cannot be performed before procurement in the DCD setting. Furthermore, current clinical protocols for evaluation of DCD hearts also raise concern. Although transplantation of viable DCD hearts procured with the normothermic regional perfusion technique relies on functional assessment of hearts in the donor,[6] this technique is not permitted in all centers.[7] The more widely permitted direct procurement and perfusion protocol relies on visual inspection, coronary flow, and lactate profiles during ESHP for graft evaluation.[8] However, current assessment on ESHP using lactate profiles is based on previous experience with DBD grafts[9] and lacks sensitivity in detecting DCD hearts suitable for transplantation.[10] Thus, there is an urgent need to identify new biomarkers for graft evaluation.

Mitochondrial damage-associated molecular patterns (mtDAMPs), which are mitochondrial components released into the extracellular space by mitochondria under conditions of cellular stress,[11] such as ischemia and reperfusion injury (IRI), can be considered promising new candidates for evaluating DCD cardiac graft quality. Recognized mtDAMPs are ATP, cardiolipin, cytochrome c (cyt c), mitochondrial DNA (mtDNA), mitochondrial transcription factor A (TFAM), N-formyl peptides (NFP), and succinate. mtDAMPs initiate innate immune responses,[12] which in the case of organ transplantation, may ultimately impair graft function and contribute to the development of primary graft dysfunction.[13] mtDAMPs may be released during different stages of DCD heart transplantation (Figure 1): (i) in the donor, systemically, as a result of hemodynamic instability and trauma[14] before circulatory arrest, and locally, during warm ischemia following withdrawal of life-sustaining therapy and circulatory arrest; and (ii) during ESHP, cardiac mtDAMPs following injury incurred with procurement, establishment of the preparation, and reperfusion.

Although mtDAMPs released into the intravascular space hold potential for graft evaluation in DCD, few investigations have been performed in this specific field. In the clinical setting, 2 studies have measured circulating mtDAMPs in DCD donors, reporting correlations between donor plasma mtDNA and allograft dysfunction in liver[15] and kidney[16] transplant recipients. Importantly, a third clinical study demonstrated that DCD lungs preserved with ex-situ organ perfusion (ESOP) release large amounts of mtDNA and that these levels correlate with the development of primary graft dysfunction after transplantation.[17] More evidence about the potential role of mtDAMPs as biomarkers of graft quality in DCD transplantation has emerged from preclinical studies. In a rat model of DCD heart transplantation, our group demonstrated that cyt c and succinate are rapidly released during the first minutes of ex situ reperfusion, correlate with subsequent functional recovery, and appear to be more sensitive indicators of IRI than conventional markers of cell death (troponin T [TnT] and lactate dehydrogenase).[18] Likewise, another preclinical study using a DCD rat model reported elevated extracellular mtDNA concentration during lung reperfusion.[19]
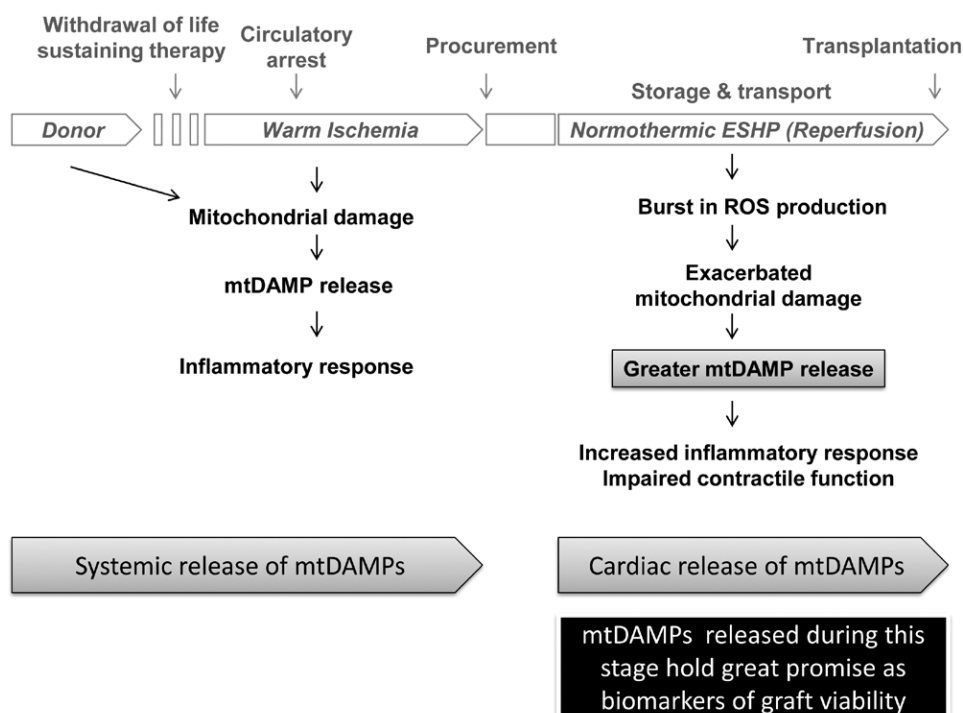
FIGURE 1. Proposed pattern of mtDAMP release in DCD heart transplantation. DCD, donation after circulatory death; ESHP, ex situ heart perfusion; mtDAMP, mitochondrial damage-associated molecular pattern; ROS, reactive oxygen species.

Whereas the use of mtDAMPs as biomarkers of graft quality in DCD heart transplantation constitutes a novel line of research, the potential of mtDAMPs as markers of cardiac damage in other models of acute ischemia, such as sudden cardiac arrest and acute myocardial infarction (AMI), has been investigated in the clinical setting. In both conditions, mtDAMPs are released into the circulation after the acute ischemic insult, similar to conditions faced in DCD heart transplantation. In light of these considerations, we propose that mtDAMPs are particularly promising candidates for biomarkers to evaluate DCD cardiac graft quality. Given the limited knowledge in this field, we submit that relevant information for DCD cardiac graft evaluation can be obtained from other acute ischemic conditions. Therefore, we performed a systematic review of original research articles in which circulating mtDAMPs were assessed in patients with cardiac arrest or AMI. The following specific questions were addressed: (1) Are circulating mtDAMPs rapidly released after an acute cardiac ischemic insult? (2) Do circulating mtDAMP levels correlate with recognized indicators of cardiac damage? and (3) Are circulating mtDAMP levels prognostic markers of survival after an episode of acute ischemia?

MATERIALS AND METHODS

A systematic literature search for original studies was performed using PubMed. Separate searches were performed for the following mtDAMPs: ATP, cardiolipin, cyt c, NFP, mtDNA, succinate, and TFAM. All PubMed searches were limited to studies performed in humans and published in English. The searches included articles until February 2, 2021.

Identification Phase

Two investigators agreed to the search terms used in PubMed to identify articles of interest. Consequently, all PubMed searches were performed with the following term: "myocardial infarction OR ischemia OR ischaemia OR cardiac arrest OR donation after circulatory death OR donation after circulatory declaration of death OR deceased organ donors," and individual searches for mtDAMPs were performed by replacing the specific mtDAMP term with the following: "(ATP OR adenosine triphosphate) AND (extracellular OR circulating)," "cardiolipin," "cytochrome c OR cyto c OR cyt c," "mtDNA OR mito-DNA OR mitochondrial DNA," "fmlp OR n-formyl peptide OR f-mit OR mitochondrial n-formyl peptides OR n-formyl peptide, mitochondrial OR n-formyl peptide, mitochondria OR mitochondrial derived formyl peptides," "succinate OR succinic acid," and "TFAM OR mitochondrial transcription factor A."

Screening Phase

The same 2 investigators separately reviewed retrieved titles and abstracts to select publications for further review according to the 3 specific questions addressed in the article. During this initial step, articles were excluded according to 2 exclusion criteria: (1) articles were preclinical studies and (2) articles reported intracellular levels of mtDAMPs. Individual publication selections were then reviewed in detail by both reviewers together to compile a final selection of publications according to the following inclusion and exclusion criteria.

Inclusion Criteria

For question 1, studies were limited to those comparing levels of circulating mtDAMPs in patients and control subjects, either healthy volunteers or patients with angina. For question 2, studies were included if correlations between circulating levels of mtDAMPs and the following biomarkers were reported: (1) myocardial damage: TnT, cardiac troponin I (cTnI) or creatine kinase MB isoform (CK-MB); (2) cardiac reperfusion injury: myocardial edema or myocardial blush grade (MBG); (3) inflammation: interleukin 6 (IL-6), tumor necrosis factor-α (TNF-α), or white blood cell count (WBC). For question 3, studies were limited to those in which survival of patients was investigated.

Exclusion Criteria

Studies were excluded according to the following exclusion criteria: (1) basic technical information was neither described in the article nor provided by authors when requested, (2) articles reported circulating mtDAMPs in clinical conditions different to myocardial infarction or cardiac arrest, (3) articles reported mtDNA contained in white blood cells, (4) articles described circulating anti-cardiolipin antibodies, and (5) clinical studies reported circulating levels of mtDAMPs that did not provide information relevant for any of the 3 specific questions.

RESULTS AND DISCUSSION

The PubMed searches retrieved 2082 articles, 18 of which were retained for detailed review. Details of the selection process are presented in Figure 2.[20]

Are Circulating mtDAMPs Rapidly Released After an Acute Cardiac Ischemic Insult?

Investigations of 5 different mtDAMPs, ATP, cardiolipin, cyt c, mtDNA, and succinate, were identified; they are described below and in Table 1.[21-29]

ATP

ATP, the main energy source in the cell, promotes inflammation and development of cardiovascular disorders through activation of the purinergic receptor P2X7 when released into the extracellular space.

A sole study has analyzed circulating levels of plasma adenylates, among them ATP, in out-of-hospital cardiac arrest (OHCA) patients after return of spontaneous circulation (ROSC).[21, 23] This study, comprising 15 OHCA patients and 8 healthy controls, reported that plasma levels of ATP were significantly higher in ischemic patients after ROSC than in healthy controls (Table 1). Interestingly, among OHCA patients, ATP levels were significantly higher in nonsurvivors than in survivors (263.3 ± 41.7 versus 153.3 ± 16.7 nmol/L; $P < 0.001$). Because of the instability of ATP, its breakdown products (ADP and AMP) were also assessed in the study. In this regard, whereas no differences were reported between healthy controls and cardiac arrest patients that survived, healthy controls presented significant lower levels of ADP and AMP than nonsurvivors after cardiac arrest. Interestingly, follow-up of survivors and nonsurvivors 24 h after resuscitation showed that the plasma level of the composite variable (ATP, ADP, and AMP) was more severely reduced in the survivor group. Whereas this innovative study[21] opens the door to further investigations into the role of ATP as a biomarker for the
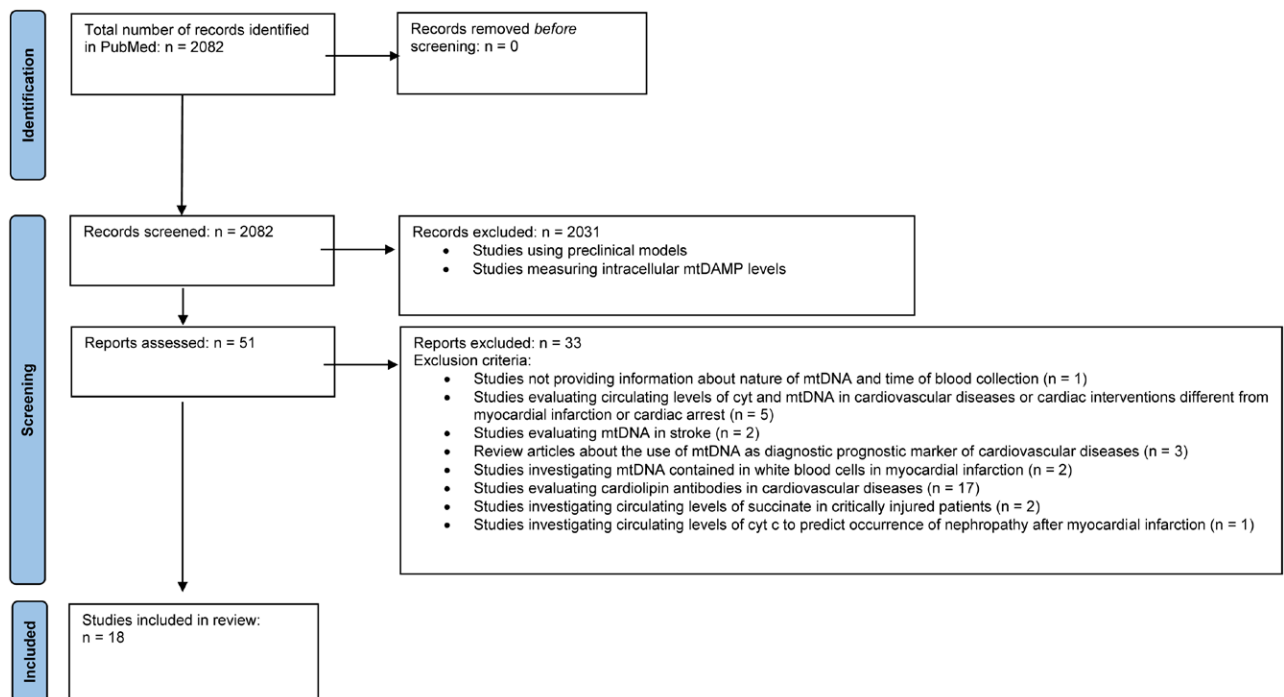
FIGURE 2. PRISMA 2020 flow diagram of excluded and included studies in the systematic review adapted from Page et al.[20] cyt, cytochrome c; mtDAMP, mitochondrial damage-associated molecular pattern; mtDNA, mitochondrial DNA; n, number of articles.

extent of acute cardiac ischemic injury, careful interpretation of these findings is required. Since OHCA patients undergo systemic ischemia and reperfusion, it is tempting to speculate that circulating ATP is released by the heart. However, ATP may be released into the circulation by other organs, such as injured brain or liver tissues. Therefore, under these conditions, circulating ATP cannot be considered a specific marker of cardiac injury.

Cardiolipin

Cardiolipin is a phospholipid exclusively found in mitochondria, predominantly in the inner mitochondrial membrane, where it helps to maintain cristae morphology and participates in the maintenance of efficient oxidative phosphorylation.

Only 1 study for cardiac arrest was retrieved from our PubMed searches.[30] In this study, a pool of brain and heart cardiolipins were identified early after cardiac arrest and, interestingly, their levels aided in the differentiation of these patients from healthy controls.[30] The study focused on brain-specific cardiolipins and showed that brain cardiolipin (70:5) can act as an indicator of neurologic injury after cardiac arrest. This study provides an impetus for investigations in the field of cardiac-specific cardiolipins as potential biomarkers of acute ischemic injury.

Cytochrome c

Cyt c functions as a shuttle for electrons in the mitochondrial respiratory chain and is physically bound to the inner mitochondrial membrane. When released into the cytosol after mitochondrial damage, cyt c activates apoptosis signaling pathways.[31] However, less is known about effects of its extracellular release.

The concept of using circulating levels of cyt c as an early marker for the diagnosis of AMI was initially explored by

Alleyne and colleagues.[32] In this first study, the authors detected cyt c in plasma of patients with AMI and healthy controls and reported a tendency toward higher cyt c levels in AMI patients.[32] This tendency was confirmed in a second study comparing AMI patients and healthy controls[23] (Table 1). Contrary to both of these studies, a third study reported no difference in serum levels of cyt c between patients with AMI before percutaneous coronary intervention (PCI) and healthy controls[22] (Table 1). Discrepant findings might be related to the differing timing of blood collection: whereas cyt c levels were elevated in AMI patients 6 h after standard therapy,[23] no differences appeared when blood samples were collected in AMI patients before administration of therapy for revascularization.[22] Strikingly, differences regarding the clearance of cyt c from the blood of AMI patients were also found between the 2 latest studies. In the study that reported higher cyt c in AMI patients than in control group, cyt c peaked after 6 h of reperfusion therapy, remained high over 24 h and disappeared after 7 d.[23] In the study that did not report any difference between AMI and control patients, cyt c levels remained high and stable from admission until the seventh day.[22]

Circulating levels of cyt c have also been analyzed in OHCA patients.[24] Significantly higher plasma levels of cyt c were measured in patients with OHCA compared with healthy controls (Table 1), consistent with results reported with AMI patients after administration of therapy.[23] Additionally, cyt c levels in patients with OHCA were significantly greater in nonsurvivors during hospitalization compared with survivors (3.7 [1.4–14.9] versus 1.3 [0.2–2.4] ng/mL; $P < 0.001$).[24] Interestingly, in survivors, cyt c levels peaked after ROSC and steadily decreased over time (12, 24, and 36 h after ROSC). In contrast, the pattern of release described in nonsurvivors differed—cyt c levels remained high and stable from ROSC for 36 h.[24]

TABLE 1.

Circulating levels of mtDAMPs in patients with AMI or cardiac arrest

mtDAMP	mtDAMP sampling	Patients with cardiac ischemia				Control patients				Units	P
		Patient population	Sample size	Central tendency	Variability	Patient population	Sample size	Central tendency	Variability		
ATP[21]	≤6 h of sustained ROSC	OHCA	102	2.2 (median)	0.7, 7.7 (IQR)	Healthy	34	0.2 (median)	0.0, 0.9 (IQR)	ng/mL	<0.001
Cyt c[22]	≤6 h of sustained ROSC	OHCA	101	1.0 (median)	0.7, 1.3 (IQR)	Healthy	46	0.8 (median)	0.5, 1.3 (IQR)	Fold change	Not significant
Cyt c[23]	≤6 h of sustained ROSC	OHCA	101	0.7 (median)	0.4, 1.1 (IQR)	Healthy	46	1.4 (median)	0.6, 2.4 (IQR)	Fold change	Not significant
Cyt c[24]	On admission (before PCI)	AMI	38	478.0 (mean)	106.0 (SD)	First onset of angina	33	157.0 (mean)	97.0 (SD)	copies/µL	<0.01
mtDNA (D-loop)[24]	Within 15 min after ROSC	OHCA	15	221.2 (mean)	26.2 (SEM)	Healthy	8	38.7 (mean)	1.2 (SEM)	nmol/L	<0.001
mtDNA (tRNAleu)[24]	6 h after standard therapy	AMI	20	0.9 (NR)	0.1 (NR)	Healthy	12	0.6 (NR)	0.1 (NR)	nmol/mL	<0.01
mtDNA (ND1)[25]	On admission (before PCI)	AMI	28	1.0 (mean)	0.4 (SD)	Healthy	30	1.2 (mean)	0.2 (SD)	ng/mL	Not significant
mtDNA (ND1)[26]	≤8 h of admission (before PCI)	AMI	25	3.7 (mean)	0.4 (SEM)	Healthy	25	0.2 (mean)	0.1 (SEM)	ng/µl	<0.05
mtDNA (COX3)[27]	On admission (post ROSC)	OHCA	20	1375.0 (mean)	375.0 (SD)	Healthy	5	112.5 (mean)	31.2 (SD)	Arbitrary units	<0.05
mtDNA (ND1)[27]	On admission (post ROSC)	OHCA	20	1500.0 (mean)	406.2 (SD)	Healthy	5	125 (mean)	62.5 (SD)	Arbitrary units	<0.05
mtDNA (ND2)[27]	On admission (post ROSC)	OHCA	20	1687.5 (mean)	562.5 (SD)	Healthy	5	112.5 (mean)	31.2 (SD)	Arbitrary units	<0.05
Succinate[28]	Not specified	AMI	3	1.0 (mean)	0.2 (SEM)	Healthy	6	Not detectable	(SEM)	mmol/L	NR
Succinate[29]	≤10 min of PCI	AMI-STEMI	115	2.7 (mean)	0.1 (SEM)	Stable angina	11	1.4 (mean)	0.1 (SEM)	µmol/L	<0.05

AMI, acute myocardial infarction; COX3, cytochrome c oxidase III; Cyt c, cytochrome c; IQR, interquartile range; mtDAMP, mitochondrial damage-associated molecular pattern; mtDNA, mitochondrial DNA; ND1, NADH dehydrogenase; ND2, NADH dehydrogenase 2; NR, not reported; OHCA, out-of-hospital cardiac arrest; PCI, percutaneous coronary intervention; ROSC, return of spontaneous circulation; STEMI, ST-segment–elevation myocardial infarction; tRNA^{leu}, transfer RNA leucine.

Collectively, findings of circulating levels of cyt c after acute cardiac ischemia support the concept that reperfusion is required either to provoke sufficient mitochondrial damage for the extracellular release of cyt c and to raise cyt c to measurable levels in the peripheral circulation.

mtDNA

Each mitochondrion contains hundreds of copies of mtDNA with the specificities of no histone packaging, unmethylated CpG motifs and, similar to bacterial DNA, recognition by Toll-like-receptor 9.

In patients with AMI, circulating mtDNA levels before PCI were increased compared with controls in 2 studies[25,26] (Table 1). Unlike cyt c, mtDNA was increased compared with controls on admission before application of PCI.[26] In both studies, mtDNA levels peaked before reperfusion interventions and subsequently decreased until reaching normal levels 2[26] or 3[25] or days after hospital admission. These findings suggest that mtDNA levels are indicative of cardiac ischemia and can be measured in blood samples even before reperfusion therapy. Differing findings between cyt c and mtDNA profiles might also result from more sensitive detection methods for DNA than for cyt c.

In cardiac arrest, contradictory findings have emerged from available studies in which different mitochondrial genes were analyzed after return of reperfusion in OHCA patients compared with healthy controls. Whereas circulating levels of 3 different mitochondrial genes (cytochrome c oxidase III, NADH dehydrogenase 1 [ND1], and NADH dehydrogenase 2) assessed early after reperfusion were significantly elevated in OHCA patients compared with healthy controls in 1 small study,[27] no differences in circulating levels of 2 mitochondrial genes (transfer RNA leucine and D-loop) assessed within 6 h ROSC between groups were observed in a larger study[24] (Table 1). Based on the higher number of patients recruited in the latter study,[24] mtDNA does not seem to be a good indicator of cardiac IRI in OHCA. However, the disagreement between studies might be related to the differing mitochondrial genes analyzed and the varied timing of mtDNA sampling. Indeed, these findings support the concept that mtDNA is an early marker of cardiac damage but quickly disappears upon reperfusion.

Succinate

Succinate is an intermediate of the Krebs cycle and, through its oxidation to fumarate, donates electrons to the mitochondrial electron transport chain. Succinate also possesses potent proinflammatory properties by binding to the G-protein–coupled receptor-91 present in dendritic cells.[33] In animal models of ischemia and reperfusion, it is widely accepted that succinate accumulates in mitochondria during ischemia and is rapidly released into the vascular space in the first minutes of reperfusion.[34] However, little is known about extracellular levels of succinate in response to acute episodes of ischemia in the clinical setting.

Three studies have investigated circulating succinate levels in AMI. In 1 small study, comparing ST-segment elevation myocardial infarction (STEMI) patients with healthy controls, circulating levels of succinate measured within 1 h after symptom onset and before reperfusion therapy were lower in patients with STEMI[35] (values not reported). In contrast, 1 very small study reported that circulating succinate was increased in AMI patients compared with healthy controls, as

succinate was detectable only in the former group[29] (Table 1). These findings agree with a larger study reporting higher circulating succinate concentrations within 10 min of PCI in patients with STEMI versus patients with angina (Table 1).[28] Taken together, these findings suggest that to reach measurable levels, succinate must be quantified in blood samples collected during the first minutes of reperfusion.

Do Circulating mtDAMP Levels Correlate With Recognized Indicators of Cardiac Damage?

Correlations between circulating cyt c, mtDNA, and succinate with recognized markers of cardiac damage were identified only in clinical studies with AMI and are described below and in Table 2.[26,28,36-38]

Cytochrome c

Two relevant studies were retrieved. In AMI patients treated with PCI, circulating cyt c and CK-MB were markedly increased post PCI, reaching their peak values approximately 12 h after PCI and gradually decreasing afterwards (measurements were started before PCI and taken every 6 h until 96 h post PCI).[36] Interestingly, CK-MB disappeared more rapidly than cyt c. Importantly, significant correlations were reported between the peak levels of both markers and between the corresponding area under the curve determinations (Table 2). Additionally, the peak value of cyt c negatively correlated with MBG (Table 2),[36] indicating that circulating cyt c levels post PCI might also provide information about vascular damage/dysfunction. Another study identified cyt c only in 37% AMI patients (280 patients out of 753) when assessed on admission before therapy[37] and reported that troponin I was significantly increased in patients with detectable cyt c compared with patients with nondetectable cyt c (0.8 [0.2–3.3] ng/mL versus 0.6 [0.1–2.1] ng/mL, $P = 0.018$). However, no significant correlation between markers was found (Table 2). Taken together, we describe significant positive correlations between markers of myocardial ischemic injury and cyt c levels, but only when this mtDAMP was assessed in blood of patients collected after the initiation of reperfusion. Therefore, these findings strengthen the concept that cyt c profiles are of greater value for evaluation of cardiac status when measured after, rather than before, reperfusion.

mtDNA

Only 2 studies were identified with our search strategy. First, a significant correlation between circulating ND1 measured 3 h post PCI and TnT peak (12 h post PCI; Table 2), was reported in AMI patients.[38] This finding reinforces the concept that ND1 is rapidly released by cardiac cells after an ischemic insult and may thus provide information about ischemic damage earlier than TnT. Second, in AMI patients before reperfusion therapy, circulating ND1 also correlated with inflammatory cytokines IL-6 and TNF-α, as well as WBC, suggesting that circulating mtDNA levels might provide information about inflammatory status (Table 2).[26] Interestingly, all correlations between circulating ND1 and inflammatory cytokines were measured in samples taken before reperfusion, which was consistent with the concept that mtDNA is rapidly released following ischemic onset.

Succinate

In 1 study with 115 STEMI patients, circulating succinate quantified within 10 min after PCI did not significantly

TABLE 2.

Correlations between circulating mtDAMPs and CMs of myocardial or coronary vascular injury or IMs

Correlations between mtDAMPs and CMs of myocardial ischemic injury							
mtDAMP	Patient population	Sample size	mtDAMP sampling	CM	CM sampling	Predictive value	Statistical analysis
Succinate[28]	AMI	115	≤10 min post PCI	Troponin AUC	Over 48 h post PCI	$\rho = 0.14$ $P = 0.27$	Spearman correlation
Cyt c[36]	AMI	55	12 h post PCI	CK-MB	9 h post PCI	$\rho = 0.35$ $P = 0.01$	Spearman correlation
Cyt c AUC[36]	AMI	55	Over 96 h after admission	CK-MB AUC	Over 96 h after admission	$\rho = 0.32$ $P = 0.02$	Spearman correlation
Cyt c[37]	AMI	280	Before reperfusion	cTnI	Before reperfusion	$r = 0.03$ $P = 0.59$	NR
mtDNA (ND1)[38]	AMI	20	3 h post PCI	TnT	12 h post PCI	$\rho = 0.53$ $P = 0.01$	Spearman correlation
Correlations between mtDAMPs and CMs of coronary vascular injury							
mtDAMP	Patient population	Sample size	mtDAMP sampling	CM	CM sampling	Predictive value	Statistical analysis
Succinate[28]	AMI	115	≤10 min post PCI	Myocardial edema	2 d post PCI	$\rho = 0.43$ $P < 0.05$	Spearman correlation
Cyt c[36]	AMI	55	12 h post PCI	MBG	NR	$\rho = -0.41$ $P < 0.05$	Spearman correlation
Correlations between mtDAMPs and IMs							
mtDAMP	Patient population	Sample size	mtDAMP sampling	IM	IM sampling	Predictive value	Statistical analysis
mtDNA (ND1)[26]	AMI	38	Before reperfusion	IL-6	Before reperfusion	$R = 0.52$ $P < 0.001$	Pearson correlation
mtDNA (ND1)[26]	AMI	38	Before reperfusion	TNF-α	Before reperfusion	$R = 0.54$ $P < 0.001$	Pearson correlation
mtDNA (ND1)[26]	AMI	38	Before reperfusion	WBC	Before reperfusion	$R = 0.435$ $P < 0.001$	Pearson correlation

AMI, acute myocardial infarction; AUC, area under the curve; cTnI, cardiac troponin I; CM, conventional marker; Cyt c, cytochrome c; CK-MB, creatine kinase—myocardial band; IL-6, interleukin 6; IM, inflammatory marker; MBG, myocardial blush grade; min, minutes; mtDAMP, mitochondrial damage-associated molecular pattern; mtDNA, mitochondrial DNA; ND1, NADH dehydrogenase; NR, not reported; PCI, percutaneous coronary intervention; R, Pearson correlation coefficient; r, correlation coefficient; ρ, Spearman correlation coefficient; TNF-α, tumor necrosis factor α; TnT, troponin T; WBC, white blood cell count.

correlate with troponin area under the curve measured over 48 h after PCI[28] but did correlate with myocardial edema assessed 2 d after PCI (Table 2).[28] These findings indicate that although circulating succinate at early reperfusion may not provide information about acute myocardial ischemic injury, it could be relevant for indicating coronary vascular injury.

Are Circulating mtDAMP Levels Prognostic Markers of Survival After an Episode of Acute Ischemia?

Investigations with cyt c and mtDNA in AMI and under conditions of cardiac arrest are described below.

Cytochrome c

In AMI patients, 2 studies have reported a prognostic value for cyt c on the incidence of major adverse cardiovascular events and mortality. In a cohort of 753 patients, circulating cyt c levels before reperfusion therapy were shown to be an independent predictor of in-hospital mortality (odds ratio, 3.03; 95% confidence interval [CI], 1.89-5.75; $P < 0.001$) and added complementary predictive value for mortality when combined with cTnI (odds ratio, 2.93; 95% CI, 1.28-6.71; $P = 0.01$).[37] A study comprising 160 patients, in which circulating cyt c was quantified after reperfusion therapy, reported that patients with higher cyt c levels had a greater incidence of circulatory death after 1 y of follow-up (log rank, $P = 0.029$).[39] Taken together, these findings suggest that cyt c determination may help in the identification of patients with AMI at high

risk for adverse outcomes and adds value to the information provided by cardiac troponins about myocardial damage.

In contrast, cyt c levels assessed within 6 h after ROSC in patients with OHCA were not associated with in-hospital mortality (odds ratio for mortality per ng/mL increase in cyt c: 1.05; 95% CI, 1.00-1.10; $P = 0.07$).[24] This might indicate that longer follow-up of OHCA patient survival is required to fully investigate the prognostic utility of cyt c.

mtDNA

A small study of 14 patients suffering from acute coronary diseases showed that those presenting 16S ribosomal RNA gene mtDNA levels higher than 4000 copies/mL upon arrival at the hospital had a death probability of 50%.[40]

In a cohort of 85 patients with OHCA, plasma ATP synthase protein 8 (mitochondrial gene) concentration at early reperfusion was a robust independent predictor of 3-d survival (odds ratio, 3.70; 95% CI, 1.45-7.32).[41]

Collectively, these findings suggest that circulating mtDNA levels may provide an indication of cardiac damage following an ischemic episode and exploration of their role as markers of survival of these patients merits further work.

mtDAMPs in DCD Heart Transplantation: Current Situation, Perspectives, and Recommendations

Despite the central role of mitochondria in IRI, few studies have investigated mtDAMPs in a clinical DCD scenario.[15-17] In

1 study, circulating levels of mtDNA and NFP were identified in living and deceased (55 DBD and 10 DCD) donors before kidney and liver procurement. Interestingly, both mtDAMPs were elevated in both types of deceased donors compared with living donors. As expected, mtDNA significantly correlated with proinflammatory cytokines (IL-6, interleukin 8, interleukin 2R, interleukin 1R, and interferon gamma), whereas NFP did not correlate with any of the proinflammatory parameters analyzed. Furthermore, early graft dysfunction following liver transplantation was associated with higher levels of circulating mtDNA in donors. A second study, performed in a cohort of 75 DCD donors, reported donor plasma mtDNA as an independent risk factor for delayed graft function in kidney recipients.[16] Finally, a retrospective clinical study in which perfusate of 27 DCD lungs were collected at 1 and 4 h of ESOP showed that circulating mtDNA level assessed at 1 h of ESOP is a good candidate to predict primary graft dysfunction in recipients.[17] Based on these studies, it appears that mtDNA levels measured in DCD donors before organ procurement and during ESOP can provide information concerning posttransplant allograft function and inflammatory status of DCD kidney,[16] liver,[15] and lung[17] recipients. Interestingly, it seems that assessment of mtDNA at an earlier time in ESOP may bring valuable information for predicting the quality of grafts after transplantation.[17]

Regarding the potential of mtDAMPs as biomarkers of DCD cardiac grafts, mtDAMP determination during ESHP holds great promise as its release is expected to reach its peak value at early after reperfusion, potentially revealing reperfusion injury. This concept is supported by preclinical findings in DCD hearts[18] and lungs.[19] This reperfusion-related specificity may confer an advantage to mtDAMPs as biomarkers over other markers of cell death (ie, cardiac troponins). Furthermore, since access to DCD hearts is often only possible at reperfusion after procurement, ESHP constitutes the perfect platform for the collection of perfusate samples at different time points enabling the monitoring of mtDAMP profiles. Likewise, this platform guarantees the cardiac specificity of these biomarkers. Additionally, information gained from sampling mtDAMPs during ESHP may be of value, not only in the evaluation of graft suitability for transplantation, but may also help to guide early posttransplant recipient therapies. Further investigation of the exact contribution that each

ventricle has in the total release of mtDAMPs could bring valuable information which further influences posttransplant cardiac function. This is of relevance since during the DCD protocol, the right ventricle is subjected to both pressure and volume overload because of hypoxic pulmonary vasoconstriction.[42] However, ongoing ESHP protocols in the clinical setting do not allow for sampling of coronary effluent separately from each ventricle. Nonetheless, preclinical studies would be of particular interest to identify the utility of this approach for sampling and may improve our precision in evaluating cardiac grafts.

With the recent developments in DCD heart transplantation, identification of mtDAMPs as biomarkers of DCD cardiac graft quality is particularly timely, with needs for both preclinical and clinical validation. Due to the scarcity of preclinical studies in the field of mtDAMPs in DCD heart transplantation, and as a first step toward clinical translation, we propose this concept based on published reports[18,19] and recommend further investigation. We specifically draw attention to the relevance of performing correlations between circulating levels of mtDAMPs assessed in donor blood and early during ESHP with recognized markers of graft quality, for example, lactate levels during ESHP and posttransplant outcomes. These studies will help to determine the utility of mtDAMPs in DCD cardiac graft evaluation.

Further steps toward clinical implementation require the development of new technologies that permit the rapid determination of mtDAMPs and standardization of assay methods (see Table 3).[15–18] Commercial kits for the detection of circulating cyt c and succinate permit their quantification in 1 and 3 h, respectively. However, fine-tuning of available kits and development of new techniques will be required to better support the use of circulating mtDAMPs in the clinic. In the case of mtDNA, identification and validation of specific mitochondrial genomic sequences that best reflect graft quality are still required. For rapid detection of ATP, further protocol establishment or development of new technologies are needed to avoid the complex techniques currently used for ATP determination, such as high-performance liquid chromatography. These aspects become relevant since DCD hearts can withstand ESHP periods of approximately 4–5 h,[3] a period of time that may be sufficient to permit determination of mtDAMPs in samples collected from donor blood or ESHP samples at early

TABLE 3.

Methods used to assess circulating levels of mtDAMPs in preclinical and clinical donation after circulatory death

Species	Organ	Sample	mtDAMP	mtDAMP sampling	Methodology	Reported units	Estimated time of analysis
Human[15]	Liver and kidney	Plasma	mtDNA (COX2)	Donor (before organ procurement)	RT-qPCR without DNA purification (Omni-KlenTaq-2 DNA Polymerase, DNA Polymerase Technology Inc)	ng/mL	3 h
Human[15]	Liver and kidney	Serum	NFP	Donor (before organ procurement)	Immunoassay (ELISA) (Human formylmethionine [fMet] ELISA Kit; MyBioSource Inc)	pg/mL	2 h
Human[16]	Kidney	Plasma	mtDNA (ND1)	Donor	DNA purification and subsequent RT-qPCR	NR	4 h
Human[17]	Lung	Steen solution	mtDNA (ND1)	ESOP at 1 and 4 h after procurement	DNA purification and subsequent RT-qPCR	copy/µL	4 h
Rat[18]	Heart	Krebs-Henseleit buffer	Cyt c	10 min after the onset of reperfusion with ESOP	Immunoassay (ELISA) (Quantikine Rat/Mouse Cytochrome c; R&D Systems)	ng/min/g wet weight	3 h
Rat[18]	Heart	Krebs-Henseleit buffer	Succinate	10 min after the onset of reperfusion with ESOP	Colorimetric assay (MAK184; Sigma)	nmol/min/g wet weight	1 h

COX2, cytochrome C oxidase subunit II; cyt c, cytochrome c; ELISA, enzyme-linked immunoassay; ESOP: ex situ organ perfusion; mtDAMP, mitochondrial damage-associated molecular pattern; mtDNA, mitochondrial DNA; ND1, NADH dehydrogenase1; NFP, *N*-formylated peptides; NR, not reported; RT-qPCR, real-time quantitative polymerase chain reaction.

time points. Based on our previous experience in determining cyt c and succinate in perfusate of DCD rat hearts,[18] we recommend assessing both mtDAMPs in arterial and venous samples collected from ESHP during the first hour at several timepoints, with special emphasis at early reperfusion times.

As promoters of innate inflammatory responses, circulating mtDAMPs released by DCD hearts during ESHP also possess great promise as therapeutic targets to promote cardioprotection and avoid deterioration of DCD hearts over long ESHP periods. Indeed, longer periods of ESOP have been associated with greater release of mtDNA in kidneys, suggesting that ESOP could provoke mitochondrial damage and disruption with the subsequent release of mtDAMPs.[43] Thus, strategies that can limit or reduce mtDAMP-induced effects may afford cardioprotection of DCD hearts.[44] Interestingly, the use of a mitochondrial-targeted fusion protein that inhibits oxidation of mtDNA and prevents release of mtDNA fragments has been successfully demonstrated as protective in a preclinical DCD lung model.[19] Moreover, in an animal model of lung IRI, treatment of lungs with apyrase (enzyme that degrades extracellular ATP) at the beginning of reperfusion significantly preserved lung function and limited formation of edema.[45] To better evaluate the clinical utility of mtDAMPs as therapeutic targets, we recommend performing preclinical studies with DCD cardiac transplantation in which perfusate solutions used for ESHP are supplemented with anti-mtDAMP therapeutic targets. We anticipate that these strategies may help to better preserve cardiac function and quality of DCD grafts and permit longer times of non-damaging ESHP.

LIMITATIONS

Translation of biomarkers validated in AMI and cardiac arrest patients into the cardiac DCD setting should be performed cautiously. We propose the novel concept of using circulating mtDAMPs measured in samples collected during ESHP of DCD hearts as prognostic markers of graft quality. Since no clinical data on mtDAMP release during ESHP of DCD hearts is currently available to our knowledge, in our article we targeted clinical conditions in which hearts are submitted to acute warm ischemia, as in DCD conditions. To do so, we reviewed available published data of circulating mtDAMPs measured in patients undergoing myocardial infarction and cardiac arrest. However, circulating levels of mtDAMPs measured in both conditions may not be fully relevant for DCD hearts during ESHP. Although circulating mtDAMP levels measured in DCD hearts during ESHP are cardiac-specific, those levels identified in AMI and cardiac arrest patients may be impacted by injury affecting not only the heart but also other organs. With our article, our goal is not to claim that circulating mtDAMPs after myocardial infarction or cardiac arrest are representative of ESHP conditions but rather to provide some evidence that supports, or does not support, investigation of mtDAMPs as biomarkers in ESHP.

Clinical protocols for the evaluation of DCD hearts

Current clinical protocols

- NRP: *in-situ* evaluation of cardiac function in donor followed by monitoring during ESHP, if required for transport

- DPP: evaluation of procured hearts during ESHP

Aspects to improve

- NRP: raises ethical concern; not universally permitted

- DPP: does not allow for functional cardiac assessment

- In both protocols: lactate levels during ESHP lack sensitivity

- Identification of new biomarkers measured during ESHP is needed

Systemic release of mtDAMPs in acute ischemic conditions: AMI and cardiac arrest

Conclusions from our systematic review

- Circulating ATP, cyt c, mtDNA and succinate are identified early after the ischemic insult

- Reperfusion may be required for effective cyt c release/assessment

- Cyt c, mtDNA and succinate levels correlate with recognized markers of myocardial and coronary vascular injury, as well as inflammatory cytokines

- Cyt c and mtDNA provide prognostic information for patient survival

mtDAMPs as biomarkers of DCD heart quality

Recommendations

- Characterize pattern of mtDAMP release in DCD donor blood and during ESHP

- Determine utility in graft evaluation and recipient outcomes

- Cutting-edge technology needs to be developed for rapid measurement of mtDAMPs in transplantation

FIGURE 3. mtDAMPs as promising biomarkers of DCD graft quality. AMI, acute myocardial infarction; cyt c, cytochrome c; DCD, donation after circulatory death; DPP, direct procurement and perfusion; ESHP, ex situ heart perfusion; mtDAMP, mitochondrial damage-associated molecular pattern; mtDNA, mitochondrial DNA; NRP, normothermic regional perfusion.

Another limitation that applies to our work is the small sample size in most reviewed articles. Except in 2 studies, which both enrolled >100 patients in the conditions reviewed, AMI[28] or cardiac arrest,[24] the numbers of patients enrolled in the remaining reviewed manuscripts was <50. Thus, although circulating mtDAMP levels appear to hold great potential as prognostic biomarkers of AMI and cardiac arrest patients, it is necessary to perform additional clinical studies comprising larger cohorts of patients before proceeding with their translation into clinical practice.

CONCLUSIONS

The main findings of our review can be summarized as follows (Figure 3): (1) ATP, cyt c, mtDNA, and succinate are released rapidly into the circulation after an acute ischemic insult; (2) circulating levels of cyt c and mtDNA positively correlate with markers of myocardial ischemic injury but only in AMI patients and only when assessed after reperfusion; (3) circulating levels of cyt c and succinate positively correlate with markers of coronary vascular injury in AMI patients after reperfusion initiation; (4) mtDNA levels assessed before reperfusion positively correlate with inflammatory markers; and (5) whereas cyt c and mtDNA exhibit prognostic value in predicting patient survival after AMI, only mtDNA seems to predict patient survival after circulatory arrest. Moreover, it seems that whereas the mtDAMP profile of release differs among specific mtDAMP molecules and ischemic conditions, all mtDAMP levels tend to peak early after the ischemic insult. This point reinforces their clinical application as early diagnosis markers of ischemic insults.

Therefore, from our systematic review, we conclude that mtDAMPs may be of particular aid in the evaluation of cardiac grafts obtained with DCD owing to their specific release after reperfusion, association with proinflammatory processes, and possibility of measurement during ESHP (Figure 3). Interestingly, mtDNA and succinate seem to be detected upon reperfusion at earlier timepoints than recognized markers of cardiac injury (see Table 2), rendering them promising early biomarkers of cardiac ischemic and reperfusion injury. However, methodology currently in use for detecting circulating mtDNA requires longer periods of time than those available for measuring circulating cyt c and succinate. Therefore, cyt c and succinate appear to be more attractive biomarkers for clinical translation in the near future. For assessment of circulating mtDAMPs of DCD hearts during ESHP, we recommend measurement in samples collected during the first hour at multiple timepoints, with special attention to early reperfusion (10–30 min). A composite quantitative index that includes multiple mtDAMP measurements may also be considered for a robust biochemical indicator of graft quality that could provide even greater value in evaluating graft suitability for transplantation.

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