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Heart failure (HF) is a global public health problem affecting an estimated 26 million people worldwide and resulting in more than 1 million hospitalizations annually in both the United States and Europe. Although the outcomes for ambulatory HF patients with a reduced ejection fraction (EF) have improved with the discovery of multiple evidence-based drug and device therapies, hospitalized heart failure (HHF) patients continue to experience unacceptably high post-discharge mortality and readmission rates that have not changed in the last 2 decades. In addition, the proportion of HHF patients classified as having a preserved EF continues to grow and may overtake HF with a reduced EF in the near future. However, the prognosis for HF with a preserved EF is similar and there are currently no available disease-modifying therapies. HHF registries have significantly improved our understanding of this clinical entity and remain an important source of data shaping both public policy and research efforts. The authors review global HHF registries to describe the patient characteristics, management, outcomes and their predictors, quality improvement initiatives, regional differences, and limitations of the available data. Moreover, based on the lessons learned, they also propose a roadmap for the design and conduct of future HHF registries. (J Am Coll Cardiol 2014;63:1123–33) © 2014 by the American College of Cardiology Foundation

Heart failure (HF) is a global public health problem affecting an estimated 26 million people worldwide. In the United States alone, the prevalence is 5.7 million, and there are 670,000 new cases/year (1–3). Among the countries represented by the European Society of Cardiology (ESC), there are an additional 15 million patients with HF (4,5). Hospitalized heart failure (HHF) is the leading cause of hospitalization in the United States and Europe, resulting in over 1 million admissions as a primary diagnosis and representing 1% to 2% of all hospitalizations (2,6–8). Although the per capita HHF rate may be beginning to decline in the United States (6,9,10) and several European nations (11–13), the early post-discharge mortality and readmission rates have remained largely unchanged and may even be worsening.

Despite pre-defined inclusion/exclusion criteria, there are major regional differences in the severity, etiology, management, and outcomes of HHF patients in international clinical trials (14). However, hospital-based registries (15–26) remain...
the primary source of real-world data on HHF. Moreover, the data collected serve to identify unmet clinical needs in order to shape public policy at all levels and guide basic, translational, and clinical research endeavors. This review will provide an overview of global HHF registries including clinical characteristics, regional variation, and limitations of the available data. We also propose a conceptual framework for the design and conduct of future HHF registries to further our understanding of HHF and inform research efforts (27,28).

Patient Demographics and Clinical Characteristics

The mean age of patients admitted with a primary diagnosis of HF ranges from 70 to 75 years, with an SD of 15 years (Table 1). Regional variation in age is likely explained by differences in the prevalence of underlying risk factors as well as the standard of living. For example, patients participating in the major North American registries tended to be older than patients enrolled in countries with developing economies. In fact, the ADHERE-AP (Acute Decompensated Heart Failure National Registry International–Asia Pacific) registry found substantial variation in age of presentation among the 8 participating countries, which showed a strong inverse correlation with the human development index, a composite measure including life expectancy, adult literacy, educational level, and standard of living (25).

Approximately 40% to 50% of HHF patients are female, a group of patients that have traditionally been underrepresented in clinical trials (29), with 1 notable exception (30). The available data suggest that a larger proportion of HHF patients in the United States are female compared with other regions of the world. This is a noteworthy observation, as female patients are unique in that they tend to be older at the time of initial diagnosis and are more likely to have heart failure with preserved ejection fraction (HFpEF) (31). However, after adjusting for differences in baseline characteristics, women have comparable outcomes to men (32). There are virtually no data on race and ethnicity outside of the United States, and when collected, these data have traditionally been limited by the accuracy and completeness of provider documentation. Despite these shortcomings, African Americans and Hispanics make up approximately 20% and 7% of HHF patients, respectively, and tend to present at a younger age and have a lower ejection fraction (EF) and higher prevalence of medical comorbidities. Although the relative burden and severity of HF is greater in these vulnerable groups, HF care and risk-adjusted outcomes appear to be equitable in the context of multiple national hospital-based registries (33–35).

An ischemic etiology is universally the most common cause of HF, whereas HF secondary to uncontrolled hypertension, valvular pathology, and congenital heart disease are likely to be more common in the developing world. Depending on how the EF is categorized (i.e., 40% to 45%) and the population under consideration, 50% to 60% of the HHF population is classified as heart failure with reduced ejection fraction (HFrEF) (36). However, hospital-based registries conducted to date have not routinely measured EF during index admission. Thus, the true epidemiologic breakdown of HF patients by EF is unknown. More importantly, it is unclear what proportion of HFpEF patients previously had a reduced EF that improved in response to evidence-based therapies. In contrast to HFrEF, HFpEF is poorly characterized as a clinical entity, and there are currently no available evidence-based therapies, although medical comorbidities should be treated accordingly (36–38). Interestingly, there are some data based on the American Heart Association’s GWTG-HF (Get With The Guidelines–Heart Failure) registry to suggest that the proportion of HHF patients classified as HFpEF is growing and may exceed HFrEF in the future (Fig. 1) (17). This may be due to increasing recognition of HFpEF by providers as well as demographic trends including aging of the population.

Importantly, cardiac and noncardiac comorbidities are extremely prevalent among HHF patients globally. More than one-half of all HHF patients have known CAD, which is complicated by myocardial infarction in 20% to 30% of cases, frequently resulting in systolic dysfunction. In addition, approximately 70% and 40% of HF patients, respectively, have a history of hypertension and atrial fibrillation, and the prevalence of these comorbidities is even higher in HFpEF where they directly contribute to diastolic dysfunction and impaired ventricular filling. Similarly, noncardiac comorbidities including diabetes mellitus, chronic kidney disease, and chronic obstructive pulmonary disease may be found in over one-third of HHF patients. These conditions not only impact the pathophysiologic progression of HF but also limit the initiation and titration of evidence-based drug and diuretic therapy.

Although regional differences in comorbid conditions likely exist, the variation is less pronounced than would be expected based on clinical trial experience (14). However, because many of these comorbidities may represent either the inclusion or the exclusion criteria for enrollment in clinical trials, it is possible that they are most accurately characterized in a trial setting. Furthermore, medical comorbidities in hospital-based studies are generally self-reported, extracted from chart review and/or billing codes, and lack formal diagnostic criteria and objective evidence of the severity. In addition, other important comorbidities among HHF patients may be systematically underappreciated due to the lack of comprehensive screening and documentation efforts in clinical practice (e.g., sleep disorders and depression). Finally, therapies that may concurrently impact the management of HF (i.e., bronchodilators in chronic obstructive
Table 1: Enrollment and Baseline Clinical Characteristics for Representative HHF Registries

<table>
<thead>
<tr>
<th>ADHERE</th>
<th>OPTIMIZE-HF</th>
<th>SWAT-HF</th>
<th>EHFS II</th>
<th>ESC-HF Pilot</th>
<th>INHF Outcome</th>
<th>EFICA</th>
<th>RO-AHFS</th>
<th>AHEAD</th>
<th>ATTEND</th>
<th>ADHERE-AP</th>
<th>ALARM-HF</th>
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<tbody>
<tr>
<td>N</td>
<td>105,388</td>
<td>48,612</td>
<td>110,621</td>
<td>3,580</td>
<td>1,892</td>
<td>581</td>
<td>3,224</td>
<td>4,153</td>
<td>4,842</td>
<td>10,171</td>
<td>4,953</td>
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<td>Age, y</td>
<td>72 ± 14</td>
<td>73 ± 14</td>
<td>74 (62-83)</td>
<td>70 ± 13</td>
<td>70 ± 13</td>
<td>72 ± 12</td>
<td>73 ± 13</td>
<td>69 ± 12</td>
<td>74 (49-88)</td>
<td>73 ± 14</td>
<td>67 (8-86)</td>
</tr>
<tr>
<td>Male</td>
<td>48</td>
<td>53</td>
<td>61</td>
<td>63</td>
<td>60</td>
<td>59</td>
<td>56</td>
<td>58</td>
<td>58</td>
<td>57</td>
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<td>—</td>
<td>15</td>
<td>44</td>
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<td>62</td>
<td>42</td>
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<tr>
<td>LVSD</td>
<td>63</td>
<td>49</td>
<td>50</td>
<td>66</td>
<td>81</td>
<td>73</td>
<td>66</td>
<td>—</td>
<td>53</td>
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<tr>
<td>LVEF, %</td>
<td>34 ± 16</td>
<td>39 ± 18</td>
<td>40 (25-55)</td>
<td>38 ± 15</td>
<td>38 ± 14</td>
<td>38 ± 15</td>
<td>38 ± 13</td>
<td>37 (16-65)</td>
<td>—</td>
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<td>54</td>
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<td>51</td>
<td>—</td>
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<td>39</td>
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<td>26</td>
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<td>34</td>
<td>45</td>
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<tr>
<td>COPD</td>
<td>31</td>
<td>15</td>
<td>19</td>
<td>19</td>
<td>30</td>
<td>—</td>
<td>10</td>
<td>—</td>
<td>22</td>
<td>21</td>
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<tr>
<td>HR, bphs/min</td>
<td>—</td>
<td>87 ± 22</td>
<td>82 (70-98)</td>
<td>95 (77-114)</td>
<td>88 ± 24</td>
<td>—</td>
<td>99 ± 29</td>
<td>90 (54-142)</td>
<td>99 ± 29</td>
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<tr>
<td>SBP, mmHg</td>
<td>144 ± 33</td>
<td>143 ± 33</td>
<td>138 (118-159)</td>
<td>135 (110-160)</td>
<td>133 ± 29</td>
<td>134 ± 33</td>
<td>126 ± 39</td>
<td>143 ± 39</td>
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<td>—</td>
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<td>—</td>
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<td>Orthopnea</td>
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<td>—</td>
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<td>63</td>
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<td>53</td>
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<td>71</td>
<td>80</td>
<td>61</td>
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<tr>
<td>BNP, pg/ml</td>
<td>840 (430-1,730)</td>
<td>832 (451-1,680)</td>
<td>821 (386-1,690)</td>
<td>870 (423-1,950)</td>
<td>1,112 (543-3,225)</td>
<td>—</td>
<td>767 (38-1,414)</td>
<td>707 (361-1,284)</td>
<td>737 ± 19</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Hgb, g/dl</td>
<td>12.1 ± 2.4</td>
<td>12.0 (10.6-13.4)</td>
<td>—</td>
<td>—</td>
<td>12.5 ± 2.1</td>
<td>—</td>
<td>13.1 ± 1.8</td>
<td>13.2 (9.6-16.2)</td>
<td>12.0 ± 2.6</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Values are %, mean ± SD, or median (25th-75th) unless otherwise specified. *30 European countries; †12 European countries; ‡Singapore, Thailand, Indonesia, Australia, Malaysia, Philippines, Taiwan, Hong Kong; §France, Germany, Italy, Spain, United Kingdom, Greece, Turkey, Australia, and Mexico. (Median/mean, 45th to 95th percentile.

ADHERE = Acute Decompensated Heart Failure National Registry; ADHERE-AP = Acute Decompensated Heart Failure National Registry International – Asia Pacific; Alt = atrial fibrillation; AHEAD = Acute Heart Failure Database; ALARM-HF = Acute Heart Failure Global Registry of Standard Treatment; ATTEND = Acute Decompensated Heart Failure Syndromes; BNP = B-type natriuretic peptide; CAD = coronary artery disease; CKD = chronic kidney disease; COPD = chronic obstructive pulmonary disease; DM = diabetes mellitus; EFICA = Epidémiologie Française de l’Insuffisance Cardiaque Aigüe; EHFS II = European Heart Failure Survey II; ESC-HF = European Society of Cardiology – Heart Failure; OPTIMIZE-HF = Get With The Guidelines-Heart Failure; PND = paroxysmal nocturnal dyspnea; RO-AHFS = Romanian Acute Heart Failure Syndromes; SBP = systolic blood pressure; SCr = serum creatinine; SAP = systolic arterial pressure; SBP = systolic blood pressure; Hgb = hemoglobin; HHF = hospitalized heart failure; HL = hyperlipidemia; HR = heart rate; Htn = hypertension; INHF = Italian Registry on Heart Failure; JVP = jugular venous pulse; LVSD = left ventricular ejection fraction; MI = myocardial infarction; OPTIMIZE-HF = Organized Program to Initiate Lifesaving Treatment in Hospitalized Patients With Heart Failure; PND = paroxysmal nocturnal dyspnea; RO-AHFS = Romanian Acute Heart Failure Syndromes; SBP = systolic blood pressure; SCr = serum creatinine;
pulmonary disease and thiazolidinediones in diabetes mellitus) have not been uniformly reported.

**Initial Clinical Presentation and Classification**

Most HHF patients are admitted for worsening chronic HF and have 1 or more identifiable acute precipitants leading to admission (Table 2). It is important to note that about one-half of patients are hypertensive at presentation. In contrast, only ~2% of patients present with an initial systolic blood pressure <90 mm Hg, suggestive of cardiogenic shock and systemic hypoperfusion. As a result, signs and symptoms of low cardiac output and inadequate end-organ perfusion (i.e., altered mentation, weak peripheral pulses, and cold clammy extremities) are relatively uncommon among HHF patients, and the vast majority present with signs and symptoms of pulmonic and systemic congestion.

Dyspnea at rest is reported in only about 34% of patients at admission; however, with provocation (i.e., exertion and orthopnea) closer to 90% of patients report some level of dyspnea (39). Rales may be appreciated on physical examination in close to 70% of patients, and two-thirds of patients have signs of systemic congestion including elevated jugular venous pressure and/or peripheral edema. Chest x-ray is obtained in more than 90% of HHF patients at initial presentation and three-quarters show radiographic evidence of pulmonary congestion.

**Laboratory, Electrocardiography, and Echocardiography Findings**

Approximately one-half of HHF patients will exhibit some degree of anemia, with over one-fourth presenting with at least moderate anemia (40). Approximately 20% of patients are hyponatremic (i.e., serum sodium <135 mEq/l) at admission, but the prevalence and degree of hyponatremia is higher and more severe in patients admitted to the intensive care unit (ICU) or coronary care unit (CCU) (41). Although about one-third of HHF patients carry a formal diagnosis of chronic kidney disease, at admission only 10% of patients will have an estimated glomerular filtration rate ≥90 ml/min/1.73 m², and about 20% will have severe renal impairment with an estimated glomerular filtration rate <30 ml/min/1.73 m² (42). Despite the fact that natriuretic peptides (NPs) have been investigated as a diagnostic marker, prognostic indicator, and potential guide to therapy, NP data in many hospital-based registries is either not included or, when it is reported, it is unavailable for a large proportion of patients.

Similarly, global HHF registry data on electrocardiographic and echocardiographic findings at admission are limited. Notably, the prevalence of atrial fibrillation on baseline electrocardiogram (ECG) may be as high as 50%, and close to 20% of HHF patients may experience new-onset atrial fibrillation during hospitalization (Table 3). In addition, after excluding ECGs with paced rhythms, more than one-third of patients have a prolonged QRS duration, a finding consistent with recent clinical trial data (43). Finally, a substantial proportion of HHF patients have normal or near-normal chamber size and systolic function and no underlying valvular heart disease, a testament to the increasing prevalence of HFpEF as well as the complex relationship among cardiac structure, function, and the clinical manifestations of HF (44) (Table 4).

**Inpatient Management**

Despite recent developments in evidence-based drug and device therapy for ambulatory HF patients with reduced EF,
there have been few advances in the management of HHF patients, of treatment still being intravenous diuretics and/or vasodilators (45–46). Although the use of diuretics is ubiquitous, the route, dose, and duration of therapy have not been routinely recorded in HHF registries (Table 3). In contrast, the utilization of vasoactive drugs (i.e., vasodilators and inotropes) exhibits substantial geographic variation and appears to play a less prominent role in HHF management in North America compared with other regions. This finding has important prognostic implications, as even short-term inotropic support has been associated with increased mortality (47).

It is also noteworthy that the vast majority of HHF patients do not receive any procedural interventions during their hospital stay. Although ischemia is by far the most common etiology of HF and many HHF patients may present with concomitant acute coronary syndrome or subclinical ischemia (48–50), <10% of patients undergo coronary angiography during index hospitalization. In addition, pulmonary artery catheters are not commonly placed during hospitalization (51).

The utilization of intravenous therapies and procedural interventions in HF patients receiving ICU/CCU-level care is likely higher, but few large observational experiences in this high-acuity population have been conducted to date.

### Utilization of Evidence-Based Therapies

HHF is an opportune time to review current management and implement evidence-based therapies for chronic HF in a controlled and monitored setting; indeed, in-hospital initiation of therapy is 1 of the best predictors of long-term use (52–54). A number of important insights can be discerned by examining the geographic variation and temporal trends in evidence-based medication utilization patterns (Fig. 2).

First, adherence to evidence-based chronic HF therapies is highest in North America, Western Europe, and Japan and lowest in the developing economies of Eastern Europe and Asia. A noteworthy exception to this generalization is that the rate of mineralocorticoid receptor antagonist (MRA) prescription at discharge is markedly lower in North America. Although the indication for MRAs has recently been expanded to include HFrEF patients spanning from mild to severe symptoms (55), providers may still be reluctant to prescribe these medications due to the risk of hyperkalemia and arrhythmic events (56). In the European Society of Cardiology Heart Failure Long-Term registry, after taking into consideration reasons for nonadherence, the real rate of undertreatment at discharge for angiotensin-converting enzyme inhibitors/angiotensin receptor blockers, beta-blockers, and MRA was estimated to be <5% (57). In addition, although only 30% of patients achieved target dosing, a specific reason was documented in close to two-thirds of patients. However, few hospital-based registries have uniformly documented medication dosing or specific intolerance(s) or relative/absolute contraindication(s) to initiating and/or up-titrating pharmacotherapy. In addition, the utilization of evidence-based medications at target dosing is likely overestimated and substantially lower at community hospitals compared with academic centers.

Many HHF patients may also be eligible for implantable cardioverter-defibrillators and/or cardiac resynchronization therapy, yet the vast majority of patients without previous implantation are discharged without being considered for device therapy. Further research is necessary to clarify the optimal timing of device implantation during hospitalization or soon after discharge among HHF patients (58–60).

### In-Hospital and Post-Discharge Outcomes

Global HHF registries show that the median length of stay (LOS) ranges from 4 to 20 days and in-hospital mortality from 4% to 30% (Fig. 3). The most recent data based on the American Heart Association’s ongoing GWTG-HF registry revealed a median LOS of approximately 4 days and in-hospital mortality of <3%. A notable outlier is the median LOS of 21 days reported in the Japanese ATTEND (Acute
Predictors of Morbidity and Mortality

The most widely accepted independent predictors of morbidity and mortality in HHF patients include age (63), cardiac (64,65) and noncardiac comorbidities (66–68), systolic blood pressure (69), renal function (i.e., blood urea nitrogen and serum creatinine) (42,69), serum sodium (41), hemoglobin (40), NP concentration (70,71), troponin (71,72), QRS duration (43,71), and evidence-based medication utilization (73,74). Data from the ADHERE (Acute Decompensated Heart Failure National Registry) (75) and OPTIMIZE-HF (69) registries both found renal function and systolic blood pressure at admission to be among the best discriminators between hospital survivors and non-survivors (Fig. 5). The OPTIMIZE-HF registry found that 8 factors, including age, weight, systolic blood pressure, sodium, serum creatinine, and comorbid disease states, could predict the combined endpoint of death or readmission with a c-index of 0.72 (76). The combination of an elevated B-type NP and positive troponin (Tn) levels (i.e., defined as TnI $\geq$1.0 ng/ml or TnT $\geq$0.1 ng/ml) in the ADHERE registry designated a small subset of patients (i.e., $\sim$5%) who were at a particularly high risk for short-term mortality (71).

Although it may be argued that the aforementioned independent predictors of morbidity and mortality and risk models may have limited external validity, much of these data have been corroborated by post-hoc analysis of global clinical trial databases. Thus, a number of important conclusions can be drawn regarding outcomes and risk prediction among HHF patients. First, HHF patients experience relatively low in-hospital mortality but are at much higher risk for early post-discharge readmission and mortality. The risk for in-hospital and post-discharge adverse events can be predicted with a high degree of fidelity by a small number of widely-available clinical variables, but a multitude of novel candidate variables may have added prognostic value (71). Risk prediction for mortality appears to be more accurate than for readmission (77). Finally, absence of high-risk features does not define an absolute low-risk population.
Impact of Quality Improvement Initiatives

Although most global HHF registries have been purely observational and have not included a prospective quality improvement component, there are some data to suggest that simply participating in a registry with intermittent benchmarked data reports can lead to improvements in quality measures and outcomes over time (78). For instance, in the ADHERE registry, over a 3-year period, beta-blocker use during hospitalization and at discharge increased by 30% and 29%, LOS decreased from 6.3 to 5.5 days, and in-hospital mortality decreased from 4.5% to 3.2%.

Following the success of the ADHERE registry, the next major North American registry, OPTIMIZE-HF, was designed to be a national quality improvement registry (79). The process-of-care improvement component included evidence-based practice algorithms, standardized order sets, discharge checklists, and a variety of other features. The OPTIMIZE-HF registry found that beta-blocker use increased from 76% to 86%, and there was a significant reduction in LOS, but there was only a trend toward lower in-hospital or post-discharge mortality, or the composite of post-discharge mortality and readmission rate (80). However, use of the process-of-care components was associated with lower risk-adjusted in-hospital mortality and post-discharge deaths and readmissions.

The OPTIMIZE-HF registry experience is further corroborated by the American Heart Association’s GWTG-HF registry quality improvement initiative, which has shown that participating U.S. hospitals have better outcomes compared with nonparticipant hospitals (81). Furthermore, among participating hospitals, those receiving awards related to achieving a certain degree of benchmark compliance had...
lower risk-adjusted HF mortality. These improved outcomes are thought to be due to better adherence to the process-of-care intervention, because award-winning and non-award-winning hospitals performed comparably on other core hospital performance measures (i.e., pneumonia and surgical infection prevention) not specifically addressed by the GWTG-HF program.

Collectively, the data suggest that it is feasible to design and conduct a quality improvement initiative in HHF on a large scale and that adopting standardized clinical decision-making tools may increase adherence to quality measures and may improve in-hospital and post-discharge outcomes. Given the ongoing high post-discharge event rate, future global HHF registries may focus on implementing interventions aimed at the transition of care from the inpatient to the outpatient setting (82-85).

Limitations of Existing Data and Future Directions

Despite the immense benefit of past HHF registries, there remain important knowledge gaps. It may be prudent to systemically discuss these shortcomings, barriers to implementation, and strategies for success. Areas to focus on in the design and conduct of future global HHF registries include, but are not limited to, geographic representation, patient enrollment, data capture, and quality improvement interventions.

The single greatest limitation of the HHF registries conducted to date is the relative paucity of data collected outside of North America and Western Europe. Thus, the current knowledge base on HHF is largely derived from a nonrepresentative sample including only slightly more than 15% of the world’s population. Expanding recruitment to other regions may require an investment in infrastructure and personnel where it is more rudimentary or nonexistent. This will require the international medical community to partner with local governments and existing organizations to achieve shared goals. A recent post-hoc analysis of the EVEREST (Efficacy of Vasopressin Antagonism in Heart Failure: Outcome Study with Tolvaptan) database found significant regional differences in clinical characteristics, management, and unadjusted outcomes, suggesting that despite restrictive inclusion and exclusion criteria, differential enrollment across geographic regions has the potential to dramatically alter the study population and potentially impact the overall response to investigational therapies (14). Thus, these efforts will prove pivotal as the current trend is toward conducting global clinical trials and in some cases, moving clinical trials entirely outside of the United States and Western Europe, where the costs and regulatory burden may be perceived to be prohibitive (86,87).
A related issue is that most HHF registry data have been based on the nonconsecutive enrollment of patients admitted with a definitive primary diagnosis of HF. Nonconsecutive enrollment may result in the differential recruitment of lower-acuity patients and may not be truly representative of “all-comers.” Future HHF registries should include on-site chart review specialists in order to prospectively enroll patients and enter data into a centrally-managed, web-based reporting system. However, even with dedicated staffing and institutional support, it may only be possible to enroll consecutive patients a few days per week, a compromise that may still be more reflective of the true epidemiology of HHF yet logistically feasible.

There is also a need to standardize the data collected, which has been subject to resource availability and physician preference in previous hospital-based registries. The study protocol of future HHF registries should include basic laboratory values, biomarkers (i.e., NPs and troponin), an ECG, and a transthoracic echocardiogram during index admission. Similarly, although the prevalence of inpatient therapies has been previously characterized, less is known about the route, dose, and duration of diuretics and other vasoactive medications in more contemporary and international HHF registries. In addition, a more detailed description including procedural interventions and the clinical indication is needed for higher-acuity patients requiring ICU or CCU care.

Similarly, global HHF registry data clearly show that the utilization of evidence-based therapies with proven mortality benefit has increased over time. However, patients may not achieve target dosing. The reason for not initiating or up-titrating these medications, including intolerance(s) and/or relative/absolute contraindication(s), should be queried in future hospital-based registries. Finally, in contrast to in-hospital outcomes, data on short- and long-term post-discharge mortality and readmissions have rarely been collected in HHF registries, although the majority of adverse events are known to occur post-hospitalization. Strategies should be developed to assess post-discharge outcomes while simultaneously allowing for consecutive enrollment. The status of readmissions and deaths may be supplemented by existing national reporting databases.

Quality improvement initiatives have rarely been included in large-scale HHF registries and universally have been limited to the timeframe between initial presentation and discharge. Process-of-care measures in future global HHF registries should focus on transitioning the success achieved during hospitalization to the ambulatory setting in order to reduce the unacceptably high post-discharge event rate. This may include early follow-up, surveillance laboratory testing (i.e., electrolytes and renal function), medication reconciliation and titration, and appropriate referrals for device (i.e., ICD and/or CRT) and other procedural interventions (i.e., coronary revascularization, ventricular assist device and/or transplant evaluation). In addition, permanent hospital-based registries are preferable to studies of a defined duration and may facilitate inclusion of an adaptive intervention in order to monitor the impact on outcomes over time.

Conclusions
There is currently an unmet critical need in HHF to design and conduct rational, global, hospital-based registries to better understand this heterogeneous patient population, inform public policy decisions, and guide basic, translational, and clinical research. It is highly desirable to develop a hospital-based registry that is global and geographically representative, employs consecutive or intermittently consecutive enrollment, and captures comprehensive and longitudinal data including hospital course and post-discharge outcomes. Future studies should also incorporate quality improvement initiatives focusing on continuity of care from initial presentation to the early post-discharge vulnerable period. In addition to traditional endpoints (i.e., hospitalization and mortality), patient-centered outcomes should be designed that comprehensively and longitudinally capture the burden of worsening HF (i.e., quality of life impairments and functional limitations). Thus, additional hospital-based registries are desperately needed to further our understanding of HHF and guide research endeavors (27,28,86,87).

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REFERENCES


Due to the aging and increasingly complex nature of our patients, frailty has become a high-priority theme in cardiovascular medicine. Despite the recognition of frailty as a pivotal element in the evaluation of older adults with cardiovascular disease (CVD), there has yet to be a road map to facilitate its adoption in routine clinical practice. Thus, we sought to synthesize the existing body of evidence and offer a perspective on how to integrate frailty into clinical practice. Frailty is a biological syndrome that reflects a state of decreased physiological reserve and vulnerability to stressors. Upward of 20 frailty assessment tools have been developed, with most tools revolving around the core phenotypic domains of frailty—slow walking speed, weakness, inactivity, exhaustion, and shrinking—as measured by physical performance tests and questionnaires. The prevalence of frailty ranges from 10% to 60%, depending on the CVD burden, as well as the tool and cutoff chosen to define frailty. Epidemiological studies have consistently demonstrated that frailty carries a relative risk of >2 for mortality and morbidity across a spectrum of stable CVD, acute coronary syndromes, heart failure, and surgical and transcatheter interventions. Frailty contributes valuable prognostic insights incremental to existing risk models and assists clinicians in defining optimal care pathways for their patients. Interventions designed to improve outcomes in frail elders with CVD such as multidisciplinary cardiac rehabilitation are being actively tested. Ultimately, frailty should not be viewed as a reason to withhold care but rather as a means of delivering it in a more patient-centered fashion.
Frailty, from the French *frêle* meaning of little resistance, is a biological syndrome that reflects a state of decreased physiological reserve and vulnerability to stressors (1). Stressors are broadly classified as acute or chronic illness (e.g., myocardial infarction) or iatrogenic (e.g., cardiac surgery). When exposed to such stressors, frail patients are at risk for marked and often disproportionate decompensation, adverse events, procedural complications, prolonged recovery, functional decline, disability, and mortality (2).

Frailty has become a high-priority theme in cardiovascular medicine due to the aging and increasingly complex nature of our patients (3). Evolving technical innovations have enabled clinicians to treat a wider array of patients with devices and procedures, many of whom were previously regarded as “ineligible” (4,5). Uncertainty regarding individual benefit from such treatments has been coupled with growing economic constraints on healthcare systems, such that the issue of appropriate patient selection has intensified. There is an unmet need to optimize resource allocation to prevent patients from receiving costly but futile interventions.

Assessment of frailty is instrumental to refine estimates of risk and guide patients toward personalized treatment plans that will maximize their likelihood of a positive outcome. For example, given 2 heart failure patients with similar chronological age and comorbidities, the presence of objectively-measured frailty alerts the clinician that 1 of the 2 patients has a substantially higher risk of mortality and major morbidity. Furthermore, the frail patient faces a higher risk from invasive procedures but also a potential benefit from interventions such as cardiac rehabilitation to counteract the physical weakness characteristic of frailty. A critical mass of clinicians, researchers, and policy makers have embraced the concept of frailty, yet the lack of a scientific road map to integrate frailty into practice has been a limiting factor.

The objectives of this state-of-the-art paper are to: 1) summarize the existing body of evidence for frailty in patients with cardiovascular disease (CVD); 2) offer a perspective on integrating frailty into current clinical practice; and 3) point out the knowledge gaps for future research.

**Pathobiology of Frailty**

Frailty biology is a field of ongoing research and debate (6). Putative mechanisms revolve around dysregulation of the immune, hormonal, and endocrine systems (7)—notably, up-regulation of inflammatory cytokines (8–10), decreased...
testosterone levels (11,12), and insulin resistance (13). This leads to a catabolic milieu, in which muscle breakdown exceeds muscle building, leading to a progressive decline in muscle mass and strength (sarcopenia) (14). Under stressed conditions, subclinical impairments are unmasked, and a vicious cycle ensues with physical inactivity and malnutrition leading to further decline (15,16) (Fig. 1).

The pathobiology of frailty and CVD shares several commonalities, particularly a consistent correlation with the inflammatory biomarkers interleukin-6 and C-reactive protein. Just as immune cells and cytokines exert nefarious effects on the arterial wall to promote atherosclerosis, so too do they impact cellular senescence and body composition to promote frailty. Moreover, by causing impairments in multiple organ systems, subclinical CVD is one of the important contributors to frailty (17). This biological link frames the epidemiological data, showing that frailty and CVD coexist in a large number of individuals (18).

**Frailty Assessment Tools**

Upward of 20 frailty tools have been developed to measure frailty (19); owing to a lack of consensus agreement, there is variability among studies and confusion on which tool to use. Most tools focus on 1 or more of the 5 core domains that define the frailty phenotype: slowness, weakness, low physical activity, exhaustion, and shrinking. Slowness is measured by a comfortable-pace gait speed test, weakness by a maximal handgrip strength test (using a dynamometer), and other domains by questionnaire or more specialized instruments. These domains may be considered individually or combined into a variety of scales (Table 1).

The Fried scale (20) encompasses slowness, weakness, low physical activity, exhaustion, and shrinking (unintentional weight loss), with $\geq 3$ of 5 criteria required for a diagnosis of frailty. This is the most frequently cited frailty scale and has been demonstrated to predict mortality and disability in large cohorts of community-dwelling elders and patients with CVD. Whether cognition and mood should be considered as the sixth and seventh domains of frailty or as modulating factors (i.e., catalyzing the transition from frailty to overt disability) remains an area of discussion (1,21).

The Short Physical Performance Battery (SPPB) (22,23) encompasses slowness, weakness, and balance. This is measured by a series of 3 timed physical performance tests (gait speed, chair rises, and tandem balance), each is scored 0 to 4 and a total score $\leq 5$ of 12 is required for a diagnosis of frailty.

In contrast to these multi-item frailty scales, 5-m gait speed, and to a lesser extent handgrip strength, has been advocated as a single-item measure of frailty (24–26) that often outperforms more elaborate and time-consuming scales. The gait speed test has been shown to have excellent inter-rater reliability (intraclass coefficient 0.88 to 0.96) and test-retest reliability (intraclass coefficient 0.86 to 0.91) (27). It is responsive to change, with meaningful improvements in gait speed (estimated at 0.05 to 0.2 m/s [28,29]) predicting positive outcomes on a population level (30) but not necessarily an individual patient level (31). The walking distance has varied between 3 and 10 m, although the distance has little effect on measured speed (32). The 5-m distance has been adopted by large registries and is a good balance between allowing patients to achieve a steady walking speed without eliciting cardiopulmonary symptoms. The short distance and comfortable pace are well below cardiopulmonary limitations, making the focus of this test different than a typical stress test or 6-min walk test.

The aforementioned tools reflect the clinical phenotype of frailty; another school of thought reflects the accumulation of deficits (33). Deficits encompass an assortment of up to 70 symptoms, signs, comorbidities, disabilities, and frailty traits, which are counted and summed. A simplified bedside version has been developed (34). The International Academy on Nutrition and Aging Frailty Task Force (35) favored the clinical phenotype approach, stating that comorbidities and disabilities should be disentangled from frailty.

Disabilities, broadly defined as difficulty or dependency in carrying out activities of daily living (ADL) or instrumental ADL, are erroneously interchanged with “frailty” in many instances. However, disability is more correctly conceptualized as an adverse outcome associated with frailty (e.g., a frail patient becomes disabled after a myocardial infarction) or as a separate entity altogether (e.g., a nonfrail patient becomes disabled after a motor vehicle accident).

Patient heterogeneity precludes the use of a “one size fits all” scale and cutoff for frailty. There is a ceiling effect when physical performance scales such as the SPPB are administered to healthier individuals (more challenging versions are available) (36), and conversely there is a floor effect when the scales are administered to debilitated hospitalized patients (up to 30% have a score of 0). Certain scales may be effective to screen for frailty, whereas others may be required to focus on specific and potentially treatable domains. There is justifiable reason to consider various scales, more/less challenging variants of such scales, or different cutoffs to define frailty depending on the population being studied.

**Frailty in CVD: Current Body of Evidence**

The prevalence of frailty in community-dwelling older adults is estimated to be 10% (37), and depending on the population studied and the frailty assessment tool used, rises
### Table 1

#### Recommended Frailty Assessment Tools

<table>
<thead>
<tr>
<th>Domain</th>
<th>Tool(s)</th>
<th>Operational Definition</th>
<th>Common Cutoffs for Frailty</th>
</tr>
</thead>
</table>
| Slowness        | 5 m gait speed test              | Patient is positioned behind start line and asked to walk at a comfortable pace past 5 m finish line; cue to trigger stopwatch is first footfall after start line and first footfall after finish line; repeated 3 times and averaged | Slow: < 0.83 m/s (>6s)  
Very slow: < 0.65 m/s (>7.7 s)  
Extremely slow: < 0.50 m/s (>10 s) |
| Weakness        | Handgrip strength test           | Patient is asked to squeeze a handgrip dynamometer as hard as possible; repeated 3 times (once with each hand and then with strongest hand); maximum value is recorded |
|                 | Knee extensor strength test      | Patient is seated on the dynamometer machine and asked to extend his/her knee against resistance; maximum isotonic force is recorded |
|                 | Low physical activity questionnaire | Many questionnaires have been validated; those that provide a measure of activity in kcal/week are recommended (e.g., Minnesota Leisure Time Activity, PASE, Paffenbarger Physical Activity Questionnaire) | Men: < 30 kg  
Women: < 20 kg |
|                 | Portable accelerometer           | Patient is asked to wear a portable accelerometer for a period of 1 to 7 days; total kcal expenditure is recorded | Frailty cutoffs not yet established |
| Exhaustion      | CES-D questionnaire              | Patient is asked 2 questions: How often in the past week did you feel like everything you did feel like an effort?/like you could not get going? (often [i.e., ≥3 days] or not often [i.e., 0–2 days]) | Positive if often is the answer to either question |
|                 | Anergia questionnaire            | Patient is asked 7 questions pertaining to lack of energy over the past month | Positive if major criterion “sits around a lot for lack of energy” + any 2 of 6 minor criteria |
| Shrinking       | Weight loss                      | Self-reported or measured unintentional weight change not due to dieting or exercise |
|                 | Appendicular muscle mass         | Measured muscle mass in arms and legs using a dual-energy x-ray absorptiometry scan | Frailty cutoffs not yet established; general cutoffs > 2 SD from controls  
Men: ≤ 7.23 kg/height in m²  
Women: ≤ 5.67 kg/height in m² |
|                 | Serum albumin                    | Measured serum albumin                                                               | ≤ 3.3 g/dl |

Continued on the next page
to 10% to 60% in older adults with CVD (18). In CVD, frailty confers a 2-fold increase in mortality, an effect that persists even after adjustment for age and comorbidities. The relevance and impact of frailty has been demonstrated across a broad spectrum, including: 1) stable CVD; 2) subclinical CVD; 3) heart failure; 4) coronary syndromes; 5) cardiac surgery; and 6) transcatheter aortic valve replacement (TAVR). These studies are outlined in Table 2 and are discussed in the following text.

**Stable CVD in the Community**

Beyond the cross-sectional association between frailty and CVD, the Women's Health Initiative Study revealed that women with coronary artery disease (CAD) were more likely to develop de novo frailty over 6 years (12% vs. 5%) (38), and the Health ABC (Health, Aging, and Body Composition) study showed that older adults with objectively-measured frailty were more likely to develop CAD events (3.6% vs. 2.8% per year) (39). Furthermore, the 3C (Three-City) Study showed that slow gait speed was highly predictive of cardiovascular mortality (hazard ratio [HR]: 2.9) but not mortality from cancer or other causes (HR: 1.0) (25). The EPESE (Established Populations for Epidemiologic Studies of the Elderly) Study similarly showed that impaired mobility was predictive of CAD-related mortality (relative risk [RR]: 1.8 to 2.2), with the RR increase being equivalent in magnitude to diabetes (40). In 2 studies focusing on peripheral arterial disease, frailty predicted cardiovascular mortality (HR: 2.6 to 11.0) more so than all-cause mortality (HR: 1.9 to 2.9) (41,42).

Studenski et al. (43) performed a patient-level meta-analysis of 9 large prospective studies and found that for every 0.1 m/s increase in gait speed, there was a 10% improvement in survival. Short-distance gait speed was a robust yet simple “indicator of vitality that integrates known and unrecognized disturbances in multiple organ systems many of which affect survival.” Those who walked at a speed of 0.8 m/s were predicted to reach an average life expectancy, whereas those who walked >1.0 m/s exceeded the average life expectancy (traffic signals at crosswalks are typically set at a pedestrian walking speed of 1.2 m/s, reflecting the expected lower limit for ambulatory citizens).

**Subclinical CVD**

Before frail patients manifest clinical CVD, they tend to exhibit subclinical cardiovascular derangements. A seminal substudy from the Cardiovascular Health Study screened for subclinical CVD in 4,735 older adults and found that those who were frail had an increased prevalence of undiagnosed/subclinical lesions: myocardial injury on echocardiography,
# Table 2

## Systematic Review of Frailty in Cardiovascular Disease

<table>
<thead>
<tr>
<th>First Author, Year (Ref. #)</th>
<th>N</th>
<th>Design</th>
<th>Frailty Tool</th>
<th>% Fail</th>
<th>Main Outcome(s) for Frail vs. Nonfrail</th>
</tr>
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<tbody>
<tr>
<td><strong>Community dwelling</strong></td>
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<tr>
<td>Studenski, 2011 (43)</td>
<td>34,485</td>
<td>Meta-analysis of elderly in the community</td>
<td>Gait speed (2.4–6 m)</td>
<td>32%</td>
<td>12-yr mortality: HR: 0.90 (95% CI: 0.89–0.91) per 0.1-m/s increase in gait speed</td>
</tr>
<tr>
<td>Dumurgier, 2009 (25)</td>
<td>3,208</td>
<td>Prospective cohort of elderly in the community</td>
<td>Fast pace gait speed (6 m)</td>
<td>Lowest third</td>
<td>5.1-yr mortality: 19% vs. 10%; HR: 1.4 (95% CI: 1.0–2.0)</td>
</tr>
<tr>
<td>Corti, 1996 (40)</td>
<td>4,116</td>
<td>Prospective, multicenter cohort of elderly in the community</td>
<td>Inability to walk 0.5 miles or 1 flight of stairs</td>
<td>25%</td>
<td>Prevalent CVD: Men 3.5%/yr vs. 1.3%/yr; RR: 1.8 (95% CI: 1.1–3.0)</td>
</tr>
<tr>
<td>Chin A Paw, 1999 (103)</td>
<td>450</td>
<td>Prospective, multicenter cohort of elderly men in the community</td>
<td>Chin A Paw scale</td>
<td>13%</td>
<td>Prevalent CVD: Men 50% vs. 18%; OR: 4.1 (95% CI: 1.8–9.4)</td>
</tr>
<tr>
<td>Klein, 2005 (104)</td>
<td>2,515</td>
<td>Prospective, multicenter cohort of elderly and nonelderly in the community</td>
<td>Klein scale (level 1–4)</td>
<td>53–64 yrs: 0.7%</td>
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<tr>
<td>Wood, 2006 (38)</td>
<td>40,657</td>
<td>Prospective, multicenter cohort of elderly women in the community</td>
<td>Modified Fried scale ≥3</td>
<td>16%</td>
<td>Prevalent frailty with vs. without CAD: 17% vs. 7%</td>
</tr>
<tr>
<td>Chaves, 2005 (105)</td>
<td>670</td>
<td>Prospective, multicenter cohort of elderly women in the community</td>
<td>Fried scale ≥3</td>
<td>14%</td>
<td>Prevalent CVD: Men 1.33 (95% CI: 1.06–1.67) per level</td>
</tr>
<tr>
<td>Bandeen-Roche, 2006 (106)</td>
<td>786</td>
<td>Prospective, multicenter cohort of elderly women in the community</td>
<td>Fried scale ≥3</td>
<td>11%</td>
<td>Prevalent CVD: Men 1.43 (95% CI: 1.13–1.82) per level</td>
</tr>
<tr>
<td>Newman, 2006 (39)</td>
<td>3,075</td>
<td>Prospective, multicenter cohort of elderly in the community</td>
<td>Gait speed (400 m)</td>
<td>N/A</td>
<td>Prevalent CVD: Men 1.50 (95% CI: 1.27–1.92) per level</td>
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<td>4-yr CAD mortality:</td>
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<td></td>
<td>Men 3.5%/yr vs. 1.3%/yr; RR: 1.8 (95% CI: 1.1–3.0)</td>
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<td></td>
<td>Prevalent CVD: Men 5.8%/yr vs. 4.5%/yr; RR: 1.2 (95% CI: 0.7–2.1)</td>
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<td></td>
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<td>Women 5.1%/yr vs. 2.5%/yr; RR: 1.6 (95% CI: 1.3–2.1)</td>
</tr>
</tbody>
</table>

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Table 2
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<table>
<thead>
<tr>
<th>First Author, Year (Ref. #)</th>
<th>N</th>
<th>Design</th>
<th>Frailty Tool</th>
<th>% Frail</th>
<th>Main Outcome(s) for Frail vs. Nonfrail</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Subclinical CVD</strong></td>
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<tr>
<td>Newman, 2001 (44) (Cardiovascular Health Study)</td>
<td>4,735</td>
<td>Cross-sectional study of elderly in the community</td>
<td>Fried scale ≥3</td>
<td>6%</td>
<td>Prevalent clinical CVD: 38% vs. 1%; OR: 2.79 (95% CI: 2.12-3.67) Prevalent subclinical CVD: RWM, LVH, pre-HTN, low ABI, carotid stenosis, silent CVA</td>
</tr>
<tr>
<td>Elbaz, 2005 (45)</td>
<td>2,572</td>
<td>Prospective, multicenter cohort of elderly and nonelderly in the community</td>
<td>Fast pace gait speed (6 m)</td>
<td>Lowest third</td>
<td>CIMT &gt;0.785 mm: mean gait speed 1.47 m/s vs. 1.61 m/s in CIMT ≤0.6 mm; OR: 1.9 (95% CI: 1.4-2.8) Carotid plaques: mean gait speed 1.50 m/s vs. 1.57 m/s in no plaque group; OR: 1.3 (95% CI: 1.0-1.7)</td>
</tr>
<tr>
<td>Singh, 2012 (41) (NHANES)</td>
<td>3,571</td>
<td>Prospective, multicenter cohort of elderly in the community, focus on those with PAD</td>
<td>Modified Fried scale ≥3</td>
<td>6.4% all</td>
<td>Prevalent frailty with vs. without PAD: 18% vs. 5%; OR: 2.31 (95% CI: 1.08-4.84) 4.9-yr mortality in PAD patients: 52% vs. 21%; HR: 2.88 (95% CI: 1.40-5.96) 4.9-yr CVD mortality in PAD patients: 29% vs. 6%; HR: 11.02 (95% CI: 3.41-35.60)</td>
</tr>
<tr>
<td>McDermott, 2008 (42) (Walking and Leg Circulation Study)</td>
<td>444</td>
<td>Prospective multicenter cohort of patients with PAD (ABI &lt;0.9)</td>
<td>Gait speed &lt;0.76 m/s (4 m); SPPB</td>
<td>Lowest quartile</td>
<td>4.8-yr mortality: HR: 1.87 (95% CI: 1.06-3.30) 4.8-yr CVD mortality: HR: 2.59 (95% CI: 1.04-6.44)</td>
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<tr>
<td><strong>Cardiac surgery</strong></td>
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<tr>
<td>Alfaiy, 2010 (63) (Frailty ABCs Study)</td>
<td>131</td>
<td>Prospective, multicenter cohort of elderly patients undergoing cardiac surgery</td>
<td>Gait speed &lt;0.83 m/s (5 m) (i.e., &gt;6 s to walk 5 m)</td>
<td>48%</td>
<td>In-hospital mortality/morbidity: 35% vs. 13%; OR: 3.05 (95% CI: 1.23-7.54) Discharge to facility: 48% vs. 20%; OR: 3.19 (95% CI: 1.40-8.41)</td>
</tr>
<tr>
<td>Alfaiy, 2012 (67) (Frailty ABCs Study)</td>
<td>152</td>
<td>Prospective, multicenter cohort of elderly patients undergoing cardiac surgery</td>
<td>Gait speed &lt;0.83 m/s (5 m)</td>
<td>48%</td>
<td>In-hospital mortality/morbidity: Gait speed: AUC 0.68 Fried: AUC 0.60 Expanded Fried: AUC 0.58 MSSA subdimensions: AUC 0.56</td>
</tr>
<tr>
<td>Lee, 2010 (64)</td>
<td>3,826</td>
<td>Retrospective cohort of elderly and nonelderly patients undergoing cardiac surgery</td>
<td>Ambulation dependence, ADL disability, or diagnosis of dementia</td>
<td>4%</td>
<td>In-hospital mortality: 15% vs. 5%; OR: 1.8 (95% CI: 1.1-3.0) 2-yr mortality: 30% vs. 11%; OR: 1.5 (95% CI: 1.1-2.2) Discharge to facility: 49% vs. 9%; OR: 6.3 (95% CI: 4.2-9.4)</td>
</tr>
<tr>
<td>Sündemann, 2011 (65)</td>
<td>400</td>
<td>Prospective cohort of elderly patients undergoing cardiac surgery</td>
<td>CAF score ≥11</td>
<td>50% (43% moderate, 8% severe)</td>
<td>30-day mortality: 10% vs. 4%; AUC 0.71</td>
</tr>
<tr>
<td>Sündemann, 2011 (66)</td>
<td>213</td>
<td>Prospective cohort of elderly patients undergoing cardiac surgery</td>
<td>CAF score ≥11</td>
<td>54% (45% moderate, 9% severe)</td>
<td>1-yr mortality: OR: 1.11 (95% CI: 1.04-1.16) per point</td>
</tr>
<tr>
<td>Robinson, 2011 (76)</td>
<td>223</td>
<td>Prospective cohort of elderly patients undergoing major surgery (34% cardiac surgery)</td>
<td>Timed up and go ≥15 s</td>
<td>30%</td>
<td>Discharge to facility: 67% vs. 8%; OR: 13.0 (95% CI: 5.1-33.0)</td>
</tr>
<tr>
<td>Lee, 2011 (107)</td>
<td>262</td>
<td>Prospective cohort of elderly patients undergoing femoral/aortic aneurysm surgery</td>
<td>Cross-sectional area of psoas muscles at L4 by computed tomography</td>
<td>N/A</td>
<td>90-day mortality: HR: 0.33 (95% CI: 0.16-0.68) per 1,000-mm² increase in muscle area 1-yr mortality: 9% tertile 1 vs. 5% tertile 3 3-yr mortality: 21% tertile 1 vs. 13% tertile 3</td>
</tr>
<tr>
<td>First Author, Year (Ref. #)</td>
<td>N</td>
<td>Design</td>
<td>Frailty Tool</td>
<td>% Fail</td>
<td>Main Outcome(s) for Frail vs. Nonfail</td>
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<tr>
<td>Rodés-Cabau, 2010 (70)</td>
<td>345</td>
<td>Retrospective, multicenter cohort of patients undergoing TAVR</td>
<td>Subjective judgment of treating physician</td>
<td>25%</td>
<td>Procedural complications: no differences except need for dialysis 7% vs. 1% (p = 0.009) 30-day mortality: 8% vs. 1% (p = 0.54) 8-month mortality: 2% vs. 2% (p = 1.00)</td>
</tr>
<tr>
<td>Ewe, 2011 (69)</td>
<td>147</td>
<td>Prospective, multicenter cohort of patients undergoing TAVR</td>
<td>Fried scale ≥3</td>
<td>33%</td>
<td>9-month mortality/morbidity: HR: 4.2 (95% CI: 2.0-8.8)</td>
</tr>
<tr>
<td>Green, 2012 (71)</td>
<td>102</td>
<td>Cross-sectional study of TAVR (83%), high-risk AVR (11%), and medically managed AS (5%)</td>
<td>Gait speed &lt;0.5 m/s (4.6 m)</td>
<td>63%</td>
<td>Prevalent ADL disability: OR: 1.52 (95% CI: 1.21-1.91) per 0.1 m/s; AUC 0.81</td>
</tr>
<tr>
<td>Green, 2012 (72)</td>
<td>159</td>
<td>Prospective cohort of patients undergoing TAVR</td>
<td>Modified Fried scale &gt;median</td>
<td>50%</td>
<td>30-day mortality/morbidity: nonsignificant 1-yr mortality: 17% vs. 7%; HR: 3.51 (95% CI: 1.43-8.62) 1-yr mortality: 17% vs. 7%; HR: 3.51 (95% CI: 1.43-8.62)</td>
</tr>
<tr>
<td>Schoenenberger, 2012 (73)</td>
<td>119</td>
<td>Prospective cohort of patients undergoing TAVR</td>
<td>In-house scale ≥3/7</td>
<td>50%</td>
<td>6-month ADL change ≥1: 31.3% vs. 12.1% (OR: 3.34 for functional decline; OR: 4.21 for functional decline or death, adjusted for STS) 6-month mortality: 18.6% vs. 3.3%</td>
</tr>
<tr>
<td>Stortecky, 2012 (74)</td>
<td>100</td>
<td>Prospective cohort of patients undergoing TAVR</td>
<td>In-house scale ≥3/7</td>
<td>49%</td>
<td>1-yr mortality: OR: 2.93 (95% CI: 0.93-9.24) 1-yr major cardiovascular and cerebral events: OR: 4.89 (95% CI: 1.64-14.60); both adjusted for STS</td>
</tr>
</tbody>
</table>

**Coronary disease**

| Purser, 2006 (57)          | 309   | Prospective cohort of elderly patients with severe CAD admitted to cardiac unit | Fried scale ≥3 Rockwood scale ≥1 Grip strength <25 kg Chair rise <7/30 s | 27%    | 6-month mortality: Fried: 12% vs. 8%; OR: 1.9 (95% CI: 0.6-6.0) Rockwood: 11% vs. 5%; OR: 1.4 (95% CI: 0.3-5.6) Gait speed: 14% vs. 4%; OR: 4.0 (95% CI: 1.1-13.8) Grip strength: 13% vs. 5%; OR: 2.7 (95% CI: 0.7-10.0) Chair rise: 12% vs. 5%; OR: 1.5 (95% CI: 0.4-5.0) |
| Ekerstad, 2011 (61)        | 307   | Prospective, multicenter cohort of elderly patients with NSTEMI admitted to cardiac or medical unit | CSHA Clinical Frailty Scale ≥5            | 49%    | 30-day mortality/morbidity: 48% vs. 27%; OR: 2.17 (95% CI: 28-36.7) 30-day mortality: 19% vs. 3%; OR: 4.7 (95% CI: 1.7-43.0) |
| Singh, 2011 (58)           | 629   | Prospective, multicenter cohort of elderly patients post-PCI            | Fried scale ≥3                           | 21%    | 3-yr mortality: 28% vs. 6%; HR: 2.74 (95% CI: 1.12-6.71) |
| Gharacholou, 2012 (80)     | 629   | Cross-sectional analysis of elderly patients post-PCI (same cohort as Singh) | Fried scale ≥3                           | 21%    | SAQ: more physical limitation and lower QOL (despite same angina frequency) SF-36: lower PCS and MCS scores |
| McNulty, 2011 (59)         | 101   | Retrospective, multicenter cohort of elderly and nonelderly patients post-left main PCI | Subjective judgment of treating physician ("cachexia/frailty") | 7%     | 1-yr mortality: unadjusted HR: 14.0 (95% CI: 5.4-36.0) |

Continued on the next page
<table>
<thead>
<tr>
<th>First Author, Year (Ref. #)</th>
<th>N</th>
<th>Design</th>
<th>Frailty Tool</th>
<th>% Fail</th>
<th>Main Outcome(s) for Fail vs. Nonfail</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cacciatore, 2005 (53)</td>
<td>120</td>
<td>Secondary analysis of cohort study of elderly patients with chronic heart failure</td>
<td>Lachs frailty staging system</td>
<td>15%</td>
<td>12-yr mortality: 94% vs. 69%; HR: 1.62 (95% CI: 1.08–2.45)</td>
</tr>
<tr>
<td>Attimir, 2005 (108)</td>
<td>360</td>
<td>Cross-sectional study of elderly patients with chronic heart failure referred to HF clinic</td>
<td>Attimir scale</td>
<td>42%</td>
<td>Prevalent frailty: 42%</td>
</tr>
<tr>
<td>Lupón, 2008 (49)</td>
<td>622</td>
<td>Prospective cohort of elderly patients with chronic heart failure referred to HF clinic</td>
<td>Attimir scale</td>
<td>40%</td>
<td>MIWHFQ: 39 vs. 19 (p &lt; 0.001) HF hospitalization: 21% vs. 13% (p = 0.01) 1-yr mortality: 17% vs. 5%; HR: 2.09 (95% CI: 1.11–3.32)</td>
</tr>
<tr>
<td>Volpato, 2008 (54)</td>
<td>92</td>
<td>Prospective cohort of acute patients admitted to hospital (64% decompensated heart failure)</td>
<td>SPPB admission, discharge</td>
<td>N/A</td>
<td>Length of stay: &gt;2.5-4 days for SPPB 0-4 on admission (&lt;0.5 day for every SPPB point)</td>
</tr>
<tr>
<td>Volpato, 2011 (55)</td>
<td>87</td>
<td>Prospective cohort of acute patients admitted to hospital (64% decompensated heart failure)</td>
<td>SPPB admission, discharge, 1 month</td>
<td>N/A</td>
<td>Incident disability: &gt;0.24 ADL limitations for SPPB 0-4 at discharge or SPPB decline at follow-up 1-yr mortality or hospitalization: 7% vs. 5%; OR: 5.38 (95% CI: 1.82–15.9) for SPPB 0-4 vs. 5-12; HR: 3.59 (95% CI: 1.20–10.0) for SPPB decline at follow-up</td>
</tr>
<tr>
<td>Chiarantini, 2010 (56)</td>
<td>157</td>
<td>Prospective, multicenter cohort of patients with decompensated heart failure discharged from cardiac unit</td>
<td>SPPB</td>
<td>51%</td>
<td>15-month mortality: SPPB 0: 62 per 100 PY; HR: 6.06 (95% CI: 2.19–16.76) SPPB 1-4: 29 per 100 PY; HR: 4.76 (95% CI: 1.63–14.02) SPPB 5-8: 17 per 100 PY; HR: 1.95 (95% CI: 0.67–5.70) SPPB 9-12: 9 per 100 PY; HR: 1 (referent)</td>
</tr>
<tr>
<td>Tjam, 2012 (109)</td>
<td>149</td>
<td>Secondary analysis of cohort study of elderly patients with chronic heart failure living in long-term care</td>
<td>RAI 2.0 scale</td>
<td>N/A</td>
<td>6-month mortality: AUC 0.87</td>
</tr>
<tr>
<td>Khan, 2013 (49) (Health ABC Study)</td>
<td>2,825</td>
<td>Prospective, multicenter cohort of elderly patients without baseline heart failure</td>
<td>Modified SPPB ≤2</td>
<td>31%</td>
<td>11-yr incidence HF; HR: 1.30 (95% CI: 1.10–1.55) *Overall incidence 15.9% or 1.8 per 100 PY</td>
</tr>
<tr>
<td>Chaudhry, 2013 (110)</td>
<td>758</td>
<td>Prospective, multicenter cohort of elderly in the community without baseline heart failure</td>
<td>Gait speed &lt; 0.8 m/s (4.6 m) Grip strength: men: &lt;28.5 kg; women: &lt;18.5 kg</td>
<td>42%</td>
<td>3.4-yr hospitalization: Gait speed: adjusted HR: 1.28 (95% CI: 1.06–1.55) Grip strength: adjusted HR: 1.19 (95% CI: 1.00–1.42)</td>
</tr>
<tr>
<td>Rozzini, 2003 (111)</td>
<td>995</td>
<td>Prospective cohort of acute patients admitted to cardiac unit</td>
<td>Barthel ADL &lt;90 MMSE &lt;1.8</td>
<td>20%</td>
<td>6-month mortality: 28% vs. 12% vs. 4% if both, either, or neither criteria present</td>
</tr>
</tbody>
</table>

AB = ankle-brachial index; ADL = activities of daily living; AS = aortic stenosis; AUC = area under curve; AVR = aortic valve replacement; CAD = coronary artery disease; CAF = Comprehensive Assessment of Frailty; CIMT = carotid intima media thickness; CSHA = Canadian Study of Health and Aging; CVA = cerebrovascular accident; CVD = cardiovascular disease; Health ABC = Health, Aging, and Body Compositions; HF = heart failure; HR = hazard ratio; HTN = hypertension; LVH = left ventricular hypertrophy; MCS = mental component summary; MIWHFQ = Minnesota Living With Heart Failure Questionnaire; MMSE = Mini-Mental Status Examination; MSSA = MacArthur Study of Successful Aging; NHANES = National Health and Nutrition Examination Survey; NSTEMI = non-ST-elevation myocardial infarction; OR = odds ratio; PAD = peripheral arterial disease; PCI = percutaneous coronary intervention; PCS = physical component summary; PY = person-years; QOL = quality of life; RAI = Resident Assessment Instrument; RR = relative risk; RWMA = regional wall motion abnormality; SAQ = Seattle Angina Questionnaire; SF-36 = Short-Form 36; SPPB = Short Physical Performance Battery; STS = Society of Thoracic Surgeons; TAVR = transcatheter aortic valve replacement.
brain infarcts on magnetic resonance imaging, abnormal ankle-brachial index, carotid stenosis, pre-hypertension, and left ventricular hypertrophy (44). A subanalysis from the 3C Study showed that those who had slow gait speed were more likely to have carotid intimal-medial thickening and silent carotid plaques (45). Subclinical CVD predisposes to “unsuccessful aging” (46), often defined as impaired physical or cognitive functioning and development of clinically manifest disease (47).

Heart Failure

Frailty is pertinent to the development, manifestations, and prognosis of heart failure. Frailty may be apparent at the myocardial organ level by predisposing patients to a greater extent of myocardial injury and, thus, clinical heart failure in response to stressors such as coronary ischemia or pressure or volume overload. Alternatively, frailty may be apparent at the global multisystem level by predisposing patients with heart failure to decompensate at a lower threshold and require more frequent hospitalizations. The person-years accrued for studies of frailty in the heart failure setting are greater than those for other cardiac conditions, involving approximately 2,300 patients with heart failure and up to 12 years of follow-up.

The Health ABC Study followed 2,825 older patients free of baseline heart failure over a period of 11 years and found that frailty (as measured by a modified SPPB) conferred a 30% higher risk of developing new heart failure (48). Excluding heart failure events in the first year did not alter the results, implying that frailty was not merely capturing undiagnosed/imminent cardiac dysfunction.

Although traditionally considered a geriatric condition, frailty was found by Lupón et al. (49) in one-third of younger patients with heart failure. Because chronic heart failure is known to perturb skeletal muscle and body composition (50,51) (giving rise to the phenotype of “cardiac cachexia” in extreme cases), it is not surprising to observe a large proportion of younger and older patients with heart failure exhibiting frailty traits.

Patients with chronic heart failure who were frail had a higher risk of mortality at 1 year (17% vs. 5%), heart failure hospitalizations (21% vs. 13%), and impaired quality of life (49). Chaudhry et al. (52) showed that slow gait speed was the most powerful predictor of hospitalizations, conferring a 30% increase; weak grip strength was also predictive, conferring a 16% increase. In a long-term study by Cacciatore et al. (53), patients with chronic heart failure who were frail had a substantially lower probability of surviving >10 years (6% vs. 31%).

Frailty is also relevant in acute decompensated heart failure. Volpato et al. (54,55) succeeded in administering the SPPB to patients with recently decompensated heart failure at different time points (shortly after admission, at discharge, and 1 month after discharge). A low SPPB score on admission was associated with prolonged length of stay, whereas a low SPPB score at discharge was associated with a higher risk of ADL disability, mortality, or readmission (odds ratio [OR]: 5.4). In a similar study by Chiarantini et al. (56), the yearly mortality rates were 62%, 45%, 17%, and 9% for SPPB scores of 0, 1 to 4, 5 to 8, and 9 to 12, respectively. The SPPB was responsive to change, with 63% improving versus 20% worsening from admission to discharge and 50% improving versus 18% worsening from discharge to 1 month.

Acute Coronary Syndromes and Percutaneous Coronary Interventions

In a seminal study of 309 elderly patients admitted to a coronary care unit and found to have multivessel CAD, Purser et al. (57) found that the prevalence of frailty varied considerably depending on the tool used: 27% with the Fried scale, 50% with gait speed <0.65 m/s, and 63% with the Rockwood scale. Each tool was associated with a trend toward increased 6-month mortality, yet only gait speed was statistically significant (OR: 4.0).

In a study of 629 elderly patients who underwent percutaneous coronary intervention at the Mayo Clinic, the prevalence of frailty was 21% with the Fried scale administered before discharge, conferring a significant increase in 3-year mortality (28% vs. 6%; OR: 2.74) (58). Similarly, “cachexia/frailty” was the most powerful predictor of 18-month mortality (HR: 14.0) (59) in a study of 111 patients undergoing percutaneous coronary intervention for unprotected left main disease in the Kaiser Permanente database.

Gharacholou et al. (60) further showed that, despite a similar severity of angina between frail and nonfrail patients, those who were frail had lower physical functioning and quality of life. Frailty exerted a greater impact on quality of life than comorbidities. Ekerstad et al. (61) explored the relationship between frailty and comorbidities in patients with non–ST-segment elevation myocardial infarction and showed that 79% of frail patients had at least 1 severe comorbidity. The OR for frailty to predict mortality was exponentially higher when the comorbidity burden was moderate to severe.

The studies of Ekerstad, Purser, and Lupón all showed that frail patients were less aggressively managed compared with their nonfrail counterparts; whether this is for better or for worse remains unclear. They were less likely to receive angiotensin-converting enzyme inhibitors (71% vs. 81%) and beta-blockers (63% vs. 80%), less likely to be admitted...
to a coronary care unit (35% vs. 54%), and less likely to be referred for cardiac catheterization (15% vs. 46%) or coronary artery bypass surgery (9% vs. 16%).

**Cardiac Surgery**

Cardiac surgery is an inherently relevant setting for frailty because surgery represents an iatrogenic physiological stressor to which the patient’s resiliency will determine their post-operative course. Surgeons have been performing de facto clinical frailty assessments termed the “eyeball test” or the “end of the bed-o-gram” for quite some time. More recently, investigators have examined the role of objective frailty tools to predict post-operative outcomes, and even the lay media has been attracted by this prospect (62). The utility of frailty to prospectively guide surgical decisions and improve outcomes has yet to be explicitly tested.

The Frailty ABCs (Frailty Assessment Before Cardiac Surgery) prospective study showed that slow 5-m gait speed was associated with a 3-fold increase in post-operative mortality or major morbidity (OR: 3.1) (63). A walking time of 6 s or longer (<0.83 m/s) was selected as the optimal cutoff based on receiver-operating characteristic analysis. Importantly, gait speed contributed incremental value above the Society for Thoracic Surgeons risk score (area under the curve 0.70 for risk score alone vs. area under the curve 0.74 for risk score plus gait speed). Patients with slow gait speed and a high risk score had a 43% incidence of mortality/morbidity, whereas those with normal gait speed and a low to intermediate risk score had a 6% incidence. There was a trend toward interaction for female patients and those undergoing aortic valve replacement (AVR), both of which had a markedly greater RR when frailty was present.

Studies by Lee et al. (64) and Sündermann et al. (65,66) showed that pre-operative frailty was associated with post-operative mortality at 30 days and 1 to 2 years. These 2 studies differed in the frailty scales used, as a result, in the reported prevalence of frailty. Lee et al. (64) retrospectively reviewed data from the Maritime Heart Center Cardiac Surgery Registry and defined frailty as ambulation dependence, ADL disability, or diagnosed dementia. This definition represented disability more than frailty and yielded a low 4% prevalence of frailty (mixed elderly and nonelderly cohort). Sündermann et al. (65,66) defined frailty as an aggregate of 35 criteria, which yielded a 50% prevalence of frailty. The data from Aflalo et al. (67) showed a 46% prevalence of frailty using gait speed versus 20% using the Fried scale and a low 5% prevalence of ADL disability, the single measure of gait speed outperformed other scales in predicting outcomes.

The presence of frank disability is infrequent in the general cardiac surgery population, in part because disabled patients are less likely to be referred for such a surgery. Therefore, disability scales for basic ADL are insensitive to screen elderly patients in this context. Higher-level disability scales such as the Nagi scale are more sensitive and better predict outcomes. An interaction between frailty and disability has been reported, with the prognostic effect of frailty diminishing in patients who have progressed to the more advanced stage of disability (67).

In addition to predicting post-operative mortality and morbidity, 3 studies showed that frail patients were less likely to be discharged home and were more likely to require rehabilitation and/or institutionalization after cardiac surgery (OR: 3.2 to 13.0).

Thus, it is evident that frail patients who undergo cardiac surgery have higher rates of post-operative mortality, morbidity, prolonged length of stay, and need for discharge to facilities. It is not evident whether frail patients who undergo less invasive intervention (or no intervention) have improved outcomes, although this is at times extrapolated. For the time being, a more prudent extrapolation may be that the risks and benefits of cardiac surgery should be carefully weighed in frail patients, ideally with a multi-disciplinary heart team, and if indicated, should proceed with thorough pre-operative optimization and heightened post-operative surveillance.

**Transcatheter Aortic Valve Replacement**

TAVR was initially developed for patients with severe aortic stenosis (AS) who were considered “too frail for surgery,” thus, the concept of frailty has been intimately linked to TAVR. Patients referred for TAVR typically have advanced age, multiple comorbidities, and a prevalence of frailty as high as 63%. Frailty is 1 of the “missing parameters” not captured by traditional risk scores (68) that are relied upon by clinicians as gatekeepers to TAVR. Few studies have been published in the past 2 years, limited to approximately 100 to 150 patients each, and larger studies are underway.

Although this was not the primary aim of their study, Ewe et al. (69) found that one-third of patients undergoing TAVR were frail according to the Fried scale and that frailty was among the most powerful predictors of death, myocardial infarction, stroke, or heart failure at 9 months (HR: 4.2). Frailty was not a significant predictor when defined according to the physician’s subjective judgment in the earlier study by Rodés-Cabau et al. (70).

Green et al. (71,72) presented the experience at Columbia University and surprisingly showed that frailty was predictive of 1-year mortality (17% if frail vs. 7% if not frail; HR 3.5) but not the composite of 30-day mortality or morbidity. The lack of 30-day event prediction was attributed to “adequacy of the standard selection process,” although it should be noted...
Frailty assessment tools (gait speed, even with adapted cutoffs, grip strength, and Fried scale) valid in this severely ill and often debilitated population or are these traits too ubiquitous, such that we should be relying on markers of more advanced frailty and frank disability (inability to walk, low albumin, ADL disability) to better discriminate risk? Second, does frailty increase the risk of short-term morbidity after TAVR (as it does in cardiac surgery) or does the less invasive nature of the transcatheter procedure mitigate this risk? In both cardiac surgery and TAVR, the rate of technical success remains high and the risk of intraprocedural mortality low in frail patients.

The Use of Frailty in Clinical Practice

There are many scenarios in day-to-day clinical practice in which frailty assessment can contribute valuable prognostic information and assist the clinicians in defining optimal care pathways for their patients. Ideally, frailty is not a reason to withhold care but rather a means of structuring care in a more patient-centered fashion.

A guiding principle is that frailty, disability, and comorbidity are inter-related but distinct entities (75). A second principle is that there is no definitive gold standard test for frailty, but rather an assortment of tools that reflect 1 or more domains of frailty. Multidomain tools do not necessarily provide incremental value above single-domain tools, and ease of implementation may be an important factor for adoption. A third principle is that frailty is a continuous spectrum, and specific cutoffs used to dichotomize frailty status in 1 group of patients may not be applicable in another group.

The tools recommended in Table 1 provide a (non-restrictive) framework to improve consistency and comparability among studies. For investigators seeking to test new or modified tools, they are encouraged to also use 1 of the recommended tools as a comparator and to confirm the findings in a validation cohort before reporting.

High-Yield Clinical Scenarios for Assessment of Frailty in Cardiovascular Medicine

Consideration for cardiac surgery. Frailty assessment tools should be employed in the pre-operative period; at a minimum, 5-m gait speed is a simple and powerful measure of frailty supported by prognostic data. However, it is premature to assume that frailty should determine eligibility for surgery at the individual patient level. Until data are available to prove a direct role for frailty in determining treatment, it is recommended to integrate frailty with other proven risk factors and risk models for decision making.

The timing of frailty assessment may be in the inpatient setting just before the surgery or in the outpatient setting, providing there is no intercurrent change or prolonged delay (arbitrarily > 1 month) between the assessment and surgery. The choice of when to assess frailty tends to be logistically driven depending on feasibility and work flow at the given center.

Pre-operative optimization via a multidisciplinary approach is key to counteract the multiple physiological impairments (e.g., cardiac, neurological, muscular, respiratory, renal) that
lead to the decreased physiological reserve characteristic of frailty (76). Establishing a heart team and involving the appropriate consultants are instrumental in this regard. Prompt recognition and treatment of complications are predominant; deconditioning and delirium are 2 complications that merit special attention because of their insidious and devastating course. Cardiac rehabilitation may potentially improve frailty, and although this has yet to be proven, may ultimately serve to facilitate surgical recovery for frail elderly patients. Patients may benefit, for example, if cardiac rehabilitation is initiated before a planned procedure and then continued afterward, with aerobic and strength training alongside nutritional and educational components.

Consideration for TAVR. Because patient selection continues to be a central and often challenging issue, there is hope that frailty can be used to pre-select high-risk patients with AS who are best served by TAVR rather than surgical AVR. Proving this hypothesis has not been straightforward, particularly because the majority of patients referred for TAVR are frail and the usefulness of frailty (or any other risk factor) becomes limited when it is endemic. Moreover, because the TAVR procedure induces less physiologic stress compared with surgery, it is unclear whether frailty will predict post-procedural outcomes similarly in TAVR and surgery.

The role of frailty assessment in TAVR programs may ultimately prove to be in identifying who is not frail and thus appropriate for conventional AVR. At the other end of the spectrum, the role of frailty assessment may be in identifying who is extremely frail and/or disabled and thus appropriate for medical management without intervention. The latter patient typically exhibits 1 or more features of cachexia, severe weakness, inability to ambulate, dementia, and ADL dependencies. Anecdotally, balloon aortic valvuloplasty has been used to allow for rehabilitation and improvements in heart failure as a bridge to TAVR.

Stable or recently stabilized heart failure or CAD. Once identified in the inpatient or outpatient setting, frail patients may be excellent candidates for cardiac rehabilitation (targeting frail patients may be 1 strategy to overcome the underuse of cardiac rehabilitation in general), longitudinal heart function clinics, and comprehensive geriatric assessment (77). The latter may include evaluation by experts in nutrition, physical function, cognition, psycho-geriatrics, and social support; each of which represents an area of potential vulnerability for frail patients and a blind spot for most cardiovascular practitioners who are not accustomed to dealing with these issues. This blind spot is increasingly being addressed at the educational level within cardiology curricula and continuing medical education programs.

Controversies and Future Research Questions

Defining the optimal tool set to measure frailty is a high priority. We must first determine whether there is incremental value in using multi-item scales such as Fried as opposed to single-item measures such as 5-m gait speed (57,67,78,79). We must also determine the appropriate cutoff for each tool and patient group, particularly for gait speed (>10 cutoffs have been proposed ranging from 0.5 to 1.0 m/s). This underscores the need to validate frailty tools and cutoffs in the population of interest rather than extrapolating results from other studies.

The ongoing FRAILTY-AVR multicenter study (NCT01845207) is comparing different frailty tools to determine which is most predictive in high-risk patients with AS undergoing AVR and TAVR. The Society for Thoracic Surgeons Adult Cardiac Surgery Database is collecting 5-meter gait speed data to define its value across a broad sample of patients undergoing cardiac surgery. The CoreValve U.S. pivotal trial and PARTNER II (Placement of Aortic Transcatheter Valves) trial have integrated frailty assessment in all eligible patients. The SILVER-AMI Trial (NCT01755052) is evaluating the impact of frailty alongside other risk factors in older adults hospitalized with acute myocardial infarction. Many other CVD trials have begun considering frailty.

There is an impetus to develop more robust frailty tools. Existing tools are limited in the measurement of physical activity and energy expenditure (80,81); portable pedometers and actigraphy-based tools are being investigated for this purpose (82). Whereas most tools capture muscle strength, muscle mass is only indirectly measured by weight loss. Weight loss is a flawed measure of muscle mass because excess adiposity may mask low muscle mass—termed "sarcopenic obesity" (83,84). In a study of elderly patients with cancer, 7.5% of patients were found to be underweight, whereas 46.8% were sarcopenic (85).

Muscle mass is a predictor of frailty and functional decline (86,87) and can be reliably measured by computed tomography, magnetic resonance, or dual-isotope x-ray absorptiometry (88).

Exciting translational research is seeking to gain mechanistic insights into the pathobiology of frailty and, in doing so, is fueling the development of frailty therapeutics and elusive frailty biomarkers. Biomarkers of senescence such as telomere length are not correlated with frailty (89). Other biomarkers have been correlated with frailty but remain nonspecific: C-reactive protein, interleukin 6, tumor necrosis factor alpha, neutrophil count, D-dimer, plasminogen activator inhibitor-1, testosterone, insulin-like growth factor-1, albumin, vitamin D, lymphocyte count, and memory/naive CD8 T-cell ratio. Thus, efforts to develop a specific
biomarker or panel of biomarkers for frailty have been unsuccessful to date (90).

With the accrual of diagnostic and prognostic data in CVD cohorts, we are now on the horizon of therapeutic trials to define how to best care for our frail cardiac patients (91,92). Interventions may be divided into those that: 1) direct frail patients toward less invasive therapeutic pathways; 2) monitor frail patients more closely to promptly detect and avert adverse events; 3) treat frail patients with therapies to improve their clinical or subclinical comorbidities; or 4) treat frail patients with therapies to reverse or reduce their intrinsic frailty.

A controversial question is to what extent a patient’s frailty is intrinsic or related to a specific comorbidity that can be treated (so-called “reversible” comorbidity-related frailty) (93). Some suggest that when the degree of frailty is out of proportion to the burden of comorbidity, it is intrinsic and less likely to improve after removal of the comorbidity. This suggestion is an oversimplification because the manifestations of frailty are not only influenced by comorbidity but also by a host of other modulating factors (e.g., cognition, mood, compliance, and social support).

The most widely studied interventions to improve frailty are exercise training, nutritional supplementation, testosterone replacement, and comprehensive geriatric assessment/management (94–99). Testosterone levels are associated with frailty (100), and the benefits of testosterone replacement appear to be consistent across sexes (96). Other interventions are aimed at improving the delivery and coordination of care for frail elders (101). Ideally, frailty should be identified before a cardiac intervention is imminent. Regardless of the intervention, the treatment of frail patients should emphasize patient-centered outcomes such as functional status and quality of life (102).

Conclusions

There is a substantial body of evidence to support the utility of frailty assessment in patients with diverse forms of CVD. The value of frailty as a prognostic marker is well demonstrated (with risk ratios that often exceed 2 and dwarf juxtaposed predictors in multivariable models). The value of frailty in guiding cardiovascular care and as a therapeutic target is beginning to emerge and should be expanded in future applications to improve patient outcomes. The frailty assessment tools outlined should facilitate this task by promoting a validated tool set that will allow us to compare and synthesize the results of different studies and provide a frame of reference when evaluating novel frailty markers.
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Key Words: cardiovascular disease • elderly • frailty.
THE PRESENT AND FUTURE

STATE-OF-THE-ART REVIEW

Evolving Therapies for Myocardial Ischemia/Reperfusion Injury

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CME Objective for This Article: After reading this article, the reader should be able to: 1) relate the importance of infarct size (amount of myocardium irreversibly injured during an ST-segment elevation acute myocardial infarction [STEMI]), and the need to find novel/better therapies able to reduce infarct size; 2) discuss the difference between ischemic and reperfusion injuries; 3) acknowledge that, on the basis of a timely reperfusion, additional interventions/therapies are needed to reduce the impact of reperfusion injury and, ultimately, infarct size; 4) discuss the global general pathways implicated in reperfusion-mediated injury; and 5) describe the main interventions holding the potential to reduce ischemia/reperfusion injury.

CME Editor Disclosure: JACC CME Editor Ragavendra Baliga, MD, FACC, has reported that he has no financial relationships or interests to disclose.

Author Disclosures: The authors have reported that they have no relationships relevant to the contents of this paper to disclose.

Medium of Participation: Print (article only), online (article and quiz).

CME Term of Approval

Issue Date: April 14, 2015
Expiration Date: April 13, 2016

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Manuscript received February 19, 2015; accepted February 22, 2015.
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ABSTRACT

The damage inflicted on the myocardium during acute myocardial infarction is the result of 2 processes: ischemia and subsequent reperfusion (ischemia/reperfusion injury). During the last 3 decades, therapies to reduce ischemic injury (mainly reperfusion strategies) have been widely incorporated into clinical practice. The remarkable reduction in death rates achieved with these therapies has resulted in a shift in emphasis from efforts to reduce mortality to a focus on tackling the downstream consequence of survival: post-infarction heart failure. Infarct size is the main determinant of long-term mortality and chronic heart failure, and thus, the possibility of limiting the extent of necrosis during an ST-segment elevation myocardial infarction is of great individual and socioeconomic value. After the great success of therapies to reduce ischemic injury, the time has come to focus efforts on therapies to reduce reperfusion injury, but in the recent few years, few interventions have successfully passed the proof-of-concept stage. In this review, we examine the past, present, and future therapies to reduce ischemia/reperfusion injury. (J Am Coll Cardiol 2015;65:1454–71) © 2015 by the American College of Cardiology Foundation.

Acute myocardial infarction presenting as ST-segment elevation (STEMI) is the result of abrupt occlusion of an epicardial coronary artery. As a result, the myocardium distal to the occlusion site becomes ischemic. Unrelieved ischemia causes permanent damage to the myocardium previously supplied by the occluded artery. Myocardium is destroyed and replaced by fibrous scar tissue. Because scar tissue does not contribute to myocardial contractile function, if the scar is large, global left ventricular (LV) contractile function is impaired, resulting in progressive chronic heart failure. After the demonstration that coronary thrombosis was the cause (not the result) of STEMI in the vast majority of cases, timely restoration of blood flow to the ischemic myocardium (reperfusion) became the standard treatment for these patients. Reperfusion was rapidly demonstrated to limit infarct size, improve long-term myocardial function, change the healing pattern of the infarcted zone, and more importantly, reduce mortality. A large body of experimental and clinical evidence supports the notion that reperfusion induces additional damage to the myocardium, known as reperfusion injury. As a result, the damage inflicted on the myocardium during an STEMI is better defined as ischemia/reperfusion (I/R) injury, the result of ischemic and reperfusion processes. Myocardial I/R injury is a complex phenomenon involving many players, all contributing to the final damage inflicted on the heart (Central Illustration).

In the present review, we describe the evolving therapies for the treatment of myocardial I/R injury. These include therapies targeting both ischemic and reperfusion damage. To explain the rationale for the quest for new and better therapies, we describe the pathophysiology of myocardial I/R injury and the translational path of research, from the pre-clinical discovery phase, through proof-of-concept clinical trials, to large trials aimed at changing clinical practice. In the context of this paper, the term myocardial infarction always refers to STEMI.

IMPACT OF STEMI IN 2015: A PARADIGM SHIFT

The incidence of STEMI in Western countries has declined during the last decades due to the progressive implementation of preventive therapies and better control of risk factors (1). Despite the progressive and gradual decrease in its incidence, STEMI remains a significant health problem, representing a major contributor to mortality/morbidity worldwide (1). As detailed later in this review, the implementation of timely reperfusion has resulted in a very significant reduction in the acute mortality associated with STEMI. Risk-adjusted in-hospital mortality has decreased from ≈20% in the late 1980s to ≈5% among STEMI patients treated in routine practice in 2008 (2), reaching a plateau thereafter (3). However, these impressive reductions in mortality rates, resulting from the widespread use of reperfusion strategies and adjuvant pharmacological therapies, have resulted in an increase in the incidence of chronic heart failure. Although this outcome might at first seem paradoxical, the explanation is simple: patients with a severely depressed cardiac function would not have survived...
the acute STEMI phase in the past, but with the advent of reperfusion, they now survive the index episode and live with a significantly damaged heart (2). In fact, STEMI is 1 of the major contributors to chronic heart failure. Post-infarction reduced left ventricular ejection fraction (LVEF) is 1 of the principal causes of chronic heart failure worldwide (4). The great success of reperfusion therapies has resulted in a paradigm shift in clinical research in the field of STEMI: attention is no longer solely aimed toward reducing mortality (already very low), but increasingly, is to tackle the downstream consequence of improved survival: post-infarction heart failure.

Successful clinical research has led to interventions for chronic heart failure (drugs and devices) that reduce long-term mortality in STEMI survivors with low LVEF (5). However, these strategies are economically costly, precluding their universal implementation (6). Chronic treatment of heart failure represents a huge socioeconomic burden on individuals and health care systems. As explained later in this paper, infarct size is the main determinant of adverse post-infarction outcomes, including heart failure (7). Therapies able to reduce infarct size are, therefore, urgently sought under the hypothesis that smaller infarctions will result in better long-term heart performance and that this hypothesis that smaller infarctions will result in better long-term heart performance and that this hypothesis will translate into fewer adverse clinical events (8,9). As we detail throughout this paper, the identification of refined or new therapies better able to reduce infarct size is a major challenge to 21st century society.

**PATHOPHYSIOLOGY OF I/R INJURY**

**GENERAL CONSIDERATIONS.** After the occlusion of an epicardial coronary artery, the myocardium previously perfused by the occluded artery is in jeopardy. The hypoperfused myocardial zone during myocardial infarction is known as the area at risk (AAR). If the coronary artery is not rapidly reperfused and no collateral circulation is present, most of the AAR becomes necrotic. Given that many patients receive timely reperfusion therapy, part of the AAR remains free of necrosis: the so-called salvaged myocardium. The typical morphological features of reperfused myocardial infarction are contraction bands, karyolysis, mitochondrial swelling and disruption, and membrane disruption in cardiomyocytes, accompanied by microvascular destruction, interstitial hemorrhage, and inflammation (10,11). Experimental studies identified the determinants of myocardial infarct size as: 1) the size of the AAR (12); 2) the duration of myocardial ischemia (13,14); 3) the amount of residual blood flow through collaterals (12,13); 4) the temperature of the tissue during ischemia; and 5) the hemodynamic situation during ischemia (15). The most notable hemodynamic parameter is heart rate, which determines not only myocardial demand, but also coronary blood flow (16); however, hemodynamics influence infarct size only to a limited degree, and infarct size is, thus, largely determined by lack of blood/energy supply and less by myocardial demand, which is significantly reduced by the regional lack of contraction (17).

The seminal studies by Maroko et al. (18) and Ginks et al. (19) 40 years ago first demonstrated that reperfusion salvages myocardium from infarction, and these studies initiated the ongoing success story of reperfusion therapy (20). The potential of reperfusion to induce additional injury secondary to the ischemic damage emerged soon afterward with the identification of stunning as a reversible form of myocardial reperfusion injury (21). Although the contribution of reperfusion injury to final infarct size has been disputed in the past, today it is accepted that reperfusion can induce additional damage to the myocardium. This view is supported by strong evidence that interventions applied at the end of the ischemic period (i.e., coinciding with reperfusion) can reduce infarct size. It was already recognized in the mid-1980s that gentle reperfusion at low pressure resulted in significantly less edema and a smaller infarct size than standard abrupt reperfusion at normal pressure (22). This idea was later developed by Zhao et al. (23), who demonstrated reduction of infarct size by brief episodes of coronary reocclusion/reflow at the time of reperfusion, a strategy called ischemic post-conditioning (24). Because these interventions are applied at the end of the ischemic period, they cannot reduce infarct size by reducing ischemic damage and, thus, must reduce reperfusion-related damage. From these observations it is clear not only that reperfusion injury contributes to infarct size, but also that all conditioning strategies that protect the myocardium and reduce infarct size act only in conjunction with eventual reperfusion (8,25,26).

**ROLE OF MICROCIRCULATION IN INFARCT SIZE AFTER STEMI.** The coronary microcirculation is a critical player in the complex phenomenon of myocardial I/R (Central Illustration). The microcirculatory network is the interface between the epicardial vessel and the cardiomyocytes. Thus, no matter how
efficiently and rapidly the blood flow is restored to the epicardial artery, if there is a microvascular obstruction (MVO) the myocardial tissue will remain without efficient perfusion. MVO (also termed the no-reflow phenomenon) during I/R is a major contributor to final infarct size and is an independent predictor of morbidity/mortality (27). The no-reflow phenomenon was first characterized by Kloner et al. (11,28) in dogs subjected to 90 min of coronary occlusion and subsequent reperfusion; the coronary microcirculation of these animals showed severe capillary damage, notably swollen and ruptured endothelial cells, and intraluminal thrombosis, and was surrounded by swollen and irreversibly injured cardiomyocytes. In the clinic, the no-reflow phenomenon is seen in 10% to 30% of patients with reperfused STEMI (29,30) despite successful recanalization of the epicardial coronary arteries; MVO in these patients is detected angiographically from slow or no reflow of contrast medium or by cardiac magnetic resonance (CMR). MVO develops within minutes of established reperfusion (31,32) and persists for at least 1 week (33,34). MVO is usually confined to the infarcted myocardium (14,28); however, the existence of no-reflow phenomena within the AAR but outside the infarcted area has not been systematically excluded. Notably, MVO can impair the washout of reduction equivalents and dehydrogenases that is mandatory for valid delineation of infarcted tissue by TTC staining, thus contributing to underestimation of infarct size (35). Reduced coronary blood flow is also observed outside of the AAR, but this does not reflect a no-reflow phenomenon, and is instead the result of reflex-mediated alpha-adrenergic coronary vasoconstriction (36-38).

A number of mechanisms have been proposed as contributors to MVO: 1) embolization of particulate debris from the ruptured culprit atherosclerotic lesion, with physical obstruction of the coronary microcirculation (39); 2) platelet and platelet/leukocyte aggregates that are released from the site of the culprit lesion, form in the coronary microcirculation, or arrive with the blood flow, where they form as part of the general inflammatory status associated with STEMI (40); 3) intense vasoconstriction induced by soluble vasoconstrictor substances released from the culprit lesion (41,42); 4) extravascular coronary microvascular compression due to edema in the surrounding myocardium (43); and 5) primary physical destruction of the capillary endothelium (28). These different mechanisms are not mutually exclusive and can act in concert, and their individual contribution to impaired myocardial reperfusion may vary temporally and spatially. Irreversible injury to cardiomyocytes and the coronary microcirculation are intimately related (44). High intramyocardial pressure, with a predominant contribution from edema, might be the principal cause of MVO in the endocardial layer, whereas microembolization might underlie infarct expansion in the border zone (45). However, there is currently no evidence to support a causal role for microvascular coronary obstruction in myocardial infarction, although it is intuitive to argue that the absence of efficient tissue perfusion in areas of MVO will maintain the muscle ischemia and thus contribute to infarct expansion.

The role of leukocytes in myocardial infarct development is contentious, and leukocyte infiltration may be more important for infarct healing and remodeling rather than the determination of infarct size (46). However, the potential contribution of intravascular leukocytes to MVO and infarct size deserves more attention. Myocardial infarction and MVO currently appear to be parallel phenomena that result from similar pathomechanisms: a primary energetic deficit and subsequent excessive formation of reactive oxygen species upon reperfusion. It is, therefore, not surprising that post-conditioning reduces not only myocardial infarct size, but also MVO (23,34).

**CARDIOMYOCYTE NECROSIS, APOPTOSIS, AUTOPHAGY, AND NECROPTOSIS: DOES MODE OF DEATH MATTER?**

Myocardial infarction has traditionally been viewed as a manifestation of necrotic cell death, but recently, different forms of cardiomyocyte death have been identified during I/R and are proposed to contribute to final infarct size.

_Necrosis_ is morphologically characterized by myofibrillar contraction bands, swollen and ruptured mitochondria, destruction of cardiomyocyte membranes, microvascular destruction, hemorrhage, and inflammation. Most of these morphological features are aggravated and are made more manifest by reperfusion (11,13,14,47,48). Necrosis is thought to result from unregulated and uncoordinated pathophysiological mechanisms. During ischemia, the developing acidosis from anaerobic glycolysis increases the influx of Na⁺ through the Na⁺/H⁺-exchanger, and intracellular Na⁺ accumulation is increased by the inhibition of Na⁺/K⁺-ATPase due to the lack of available ATP (49,50). The subsequent exchange of Na⁺ for Ca²⁺ by reverse mode operation of the sarcolemmal Na⁺/Ca²⁺-exchanger induces intracellular Ca²⁺ overload. Upon reperfusion, the rapid normalization of pH and re-energization in the context of elevated cytosolic Ca²⁺ induces oscillatory release and reuptake of Ca²⁺ into the sarcoplasmic reticulum, causing uncontrolled excess myofibrillar hypercontraction (50-52). The
normalization of the acidic pH also activates calpain, which digests the cytoskeleton and the sarcolemma (53). The high cytosolic concentrations of Na⁺ and Ca²⁺ result in intracellular edema when extracellular osmolarity is rapidly normalized by reperfusion. Finally, excess formation of reactive oxygen species contributes to sarcosomal disruption (54). Necrosis is typically followed by an inflammatory response.

Unlike necrosis, apoptosis, autophagy, and necroptosis are regulated processes with specific underlying signal transduction mechanisms (55,56). Apoptosis is an energy-consuming form of cell death characterized by characteristic deoxyribonucleic acid strand breaks that are identified by deoxyribonucleic acid ladder and/or terminal deoxynucleotidyl transferase dUTP nick-end labeling staining (57). Apoptosis can be initiated extrinsically by activation of sarcosomal receptors, notably FAS and tumor necrosis factor α receptors (58), or intrinsically by mitochondrial release of cytochrome c, which initiates a cascade of caspase activation leading to intracellular proteolysis, typically without an inflammatory response (56). A pivotal event in the initiation of apoptotic cell death is the opening of the mitochondrial permeability transition pore (MPTP) (59).

The MPTP is a large-conductance megachannel, which is closed under physiological conditions but opens in response to increased concentrations of calcium, inorganic phosphate, or reactive oxygen species and to a decreased inner mitochondrial membrane potential, all of which are present in myocardial I/R (60,61). Formation and opening of the MPTP results in mitochondrial matrix swelling, ultimately leading to rupture of the outer membrane and release of cytochrome c to the cytosol, where it activates the caspase cascade. Proapoptotic and antiapoptotic proteins of the Bcl-family interact with the MPTP (62). Recently, the traditional view of the MPTP has been questioned, because all of its purported constituents are dispensable under some conditions, and it is possible that the MPTP originates from F-ATP synthase (63).

Autophagy is a regulated process of lysosomal degradation and recycling of proteins, including mitochondrial proteins (mitophagy) (64). Autophagy is characterized by the presence of double-membrane vesicles (autophagosomes) and increased expression of beclin-1, light chain 3, the autophagy-related gene 5-12 complex, p62, and parkin, the last 2 of which are essential for mitophagy (65). Somewhat paradoxically, cell death by autophagy is considered protective rather than detrimental (66). For example, in pigs subjected to 45 min of coronary occlusion and reperfusion, the purported autophagy inducer chloramphenicol reduced infarct size (67). However, the role of autophagy in myocardial I/R injury in humans remains contentious (68,69).

Necroptosis shares features with necrosis and apoptosis, but is distinctly regulated by activation of receptor-interacting protein kinases 1 and 3 (70) and can be inhibited by substances such as necrostatin (71).

It is currently unclear to what extent necrosis, apoptosis, autophagy, and necroptosis are mutually exclusive processes and to what extent each contributes to infarct size. Typical features of apoptosis (terminal deoxynucleotidyl transferase dUTP nick-end labeling staining) and autophagy (characteristic protein expression) are both found in the TTC staining-defined infarct zone, which has traditionally been considered necrotic. The opening of the MPTP appears to be decisive for necrosis, apoptosis, and

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**CENTRAL ILLUSTRATION**

Myocardial ischemia/reperfusion injury is a complex phenomenon in which many players contribute to the final damage inflicted to the myocardium. 1. The first critical player is the epicardial artery. Atherosclerotic plaque rupture with superimposed thrombus results in an abrupt stop of oxygen and nutrient supply distal to the occlusion site. The opening of the epicardial vessel by mechanical or pharmacological means, as well as the thrombus burden reduction by adjuvant antiplatelet/anticoagulant therapies is only the first step toward the salvage of myocardium. During the reperfusion process (either if it is mechanical by primary angioplasty or pharmacological by thrombolitics), thrombus material and other plaque debris can be distally embolized contributing to microvascular obstruction. 2. Circulating cells contribute to the damage inflicted to the myocardium: activated platelet and leukocytes in the bloodstream not only contribute to the thrombus generation, but also can form plugs that can embolize distally into the microcirculation upon resting blood flow across the culprit lesion (a process independent from plaque debris microembolization). 3. The microcirculation (net of capillaries) is a critical player in the fate of the myocardium during ischemia/reperfusion. Once the epicardial vessel flow is restored, efficient tissue perfusion is dictated mainly by the microcirculation. Plaque debris and platelet/neutrophil aggregates can induce a mechanical obstruction of the microcirculation precluding efficient tissue perfusion. The generation of tissue edema following reperfusion can result in external compression of the microcirculation, reducing the perfusion capacity of the capillary network (double arrows). Finally, the microcirculation can disintegrate due to the previous damage and allow the leakiness of circulating cells into the interstitial space. 4. Red blood cell deposits (hemorrhage) are especially harmful due to the release of iron, contributing to the subsequent inflammatory reaction. 5. Cardiomyocytes that have survived the ischemic phase suffer during the reperfusion period due to several intracellular pathways triggered at reperfusion (see text for detailed information about these processes). 6. After the entire ischemia/reperfusion insult has passed, the significant infiltration of myocardial tissue by inflammatory cells can induce an additional damage to the myocardium.
necroptosis, and mitochondria are also decisive in mitophagy/autophagy. The importance of regulated forms of cardiomyocyte cell death in I/R injury is probably related more to their specific signal transduction mechanisms. Recognition of the different modes of cardiomyocyte death during infarction suggests the possibility of identifying therapeutic targets that can modulate these processes. From the clinical perspective, cardiomyocyte death is equally relevant whatever the mechanism.

**PRE-CLINICAL MODELS OF MYOCARDIAL I/R INJURY**

In a typical clinical scenario, a patient of middle-advanced age has various risk factors, comorbidities, and comedicated (72) and has a coronary circulation that has already undergone functional and structural remodeling before STEMI (73). The affected individual may or may not have a developed collateral circulation (74), a history of episodes of prodromal angina that may induce protection by ischemic preconditioning (75,76), or prior coronary microembolization that has induced patchy microinfarcts (39). STEMI is usually initiated by the sudden rupture of an atherosclerotic plaque and more-or-less complete occlusion of an epicardial coronary artery, most often followed by spontaneous or interventional reperfusion, but sometimes occurring without reperfusion (17). Because all of these factors contribute to the final infarct size, it is clear that no animal model can recapitulate a clinical scenario exactly. However, most of our knowledge about myocardial infarction is derived from studies in anesthetized, young, healthy animals subjected to sudden coronary occlusion and reperfusion. Animal models are critical to our understanding of the pathophysiology of human conditions, but the information obtained with any particular model can only solve a part of the puzzle. There is thus no superior animal model, and each has its uses. Rodent models help to identify potential mechanisms, but their huge anatomic/physiological differences from humans make it imprudent to extrapolate results to the clinical setting. Pilot clinical trials are justified only when solid and incontestable benefits are found in large-animal models with a much closer match to human anatomy and physiology. The history of cardiovascular medicine is littered with failed clinical experiences caused by taking shortcuts from this translational path.

**VALUE OF SMALL ANIMAL MODELS OF MYOCARDIAL INFARCTION.** Mice are increasingly used in experimental infarct studies for their ease of breeding, their low cost, and the availability of transgenic models. But, translation of results is highly limited because of their high heart rate (an order of magnitude higher than that of humans) and the small size of their hearts, which ensures that, during permanent coronary occlusion, the inner myocardial layers continue to be served with oxygen and nutrients by diffusion. Thus, infarction in mice develops within 45 to 90 min (77,78), but no more than 70% of the AAR is infarcted even with permanent coronary occlusion (79). Rodents generally have a higher heart rate than larger mammals, but whereas rats and rabbits have little collateral blood flow and fast infarct progression (80), guinea pigs have substantial collateral blood flow and very little infarct progression (81). Significant differences in the response to myocardial infarction are also found across different mice strains. Depending on the genetic background, infarct size after the same procedure can vary by 30% (82).

**LARGE ANIMAL MODELS OF MYOCARDIAL INFARCTION.** Large animals are the obligatory step before initiating human trials. Among the larger mammals, pigs and primates have little collateral blood flow, whereas cats and especially dogs have a sizeable innate collateral circulation (81). In pigs, infarction starts after 15 to 35 min of coronary occlusion and spreads such that, after 60 to 180 min, infarction is complete and affects more than 80% of the AAR (83,84). Tolerance to I/R varies notably across different pig strains. Primates show a surprising resistance to infarction, with little or no infarction evident after 40 to 60 min of coronary occlusion, and even after 90 min of occlusion, the infarct size is smaller than that in pigs (85). In dogs, infarction is largely subendocardial after 40 min of coronary occlusion and progresses to affect about 70% of the AAR after 6 h, but even permanent occlusion leaves a small zone of viable myocardium in the subepicardium (13,14). Given the variable but significant collateral blood flow in dogs, infarct size is best quantified as a fraction of the AAR and by its inverse relation to the residual blood flow (17). Notwithstanding the confounders detailed previously (age, comorbidities, comedication), infarct development in humans appears to be slower than in these large mammals. From contrast-enhanced CMR analysis (86-88) and the amount of salvageable ischemic myocardium at the time of reperfusion (89,90), one can grossly estimate that about 30% to 50% of the AAR remains viable after 4 to 6 h from the onset of anginal symptoms. Even after 12 h of coronary occlusion, interventional reperfusion can significantly limit infarct size (91). It is currently unclear whether the slower infarct progression in humans than in larger mammals is
related to a developed collateral circulation close to that in dogs (74), a species-specific greater resistance to infarction as in primates (85), pre-infarction anginal episodes that protect by ischemic pre-conditioning (76), some degree of short-term hibernation with contractile and metabolic adaptation to the reduced blood flow (92,93), or background medication, notably with platelet inhibitors (94).

Aside from the differences between animal models, there are other important factors that must be considered when performing pre-clinical studies and, more importantly, when comparing results from different models or even different laboratories. For example, the time of the day at which I/R occurs has a significant influence in the tolerance of the heart to I/R. After the initial demonstration in mice, this phenomenon has also been observed in patients (95). Similarly, the season and even the day of the week might have an effect on the results observed in animal models and, eventually, in the response to protective therapies.

**THERAPIES TO REDUCE ISCHEMIC INJURY**

Four landmark studies published more than 30 years ago, 2 experimental and 2 clinical, changed the course of STEMI treatment in less than a decade, leading the huge development of strategies to reduce ischemic injury.

1. The demonstration of a spatial progression of necrosis during an infarction. Reimer et al. (13,14) subjected anesthetized dogs to coronary occlusion of various duration and reported the progression of a wave front of myocardial necrosis from the central subendocardial layers, where ischemia is most severe, to the less ischemic lateral boundaries of the AAR and the subepicardium.

2. The demonstration of the reduced infarct size with reperfusion. Maroko et al. (18) and Ginks et al. (19) first demonstrated the existence of myocardial salvage by reperfusion at 3 h after coronary occlusion (“time is muscle”).

3. The unequivocal demonstration that coronary thrombosis is present in most cases of ongoing STEMI. DeWood et al. (96) reported detailed angiographic findings in patients studied shortly after the onset of STEMI.

4. The first experience with the administration of intracoronary thrombolytics (streptokinase), as reported by Chazov et al. (97) and later, in a larger group of patients, by Rentrop et al. (98).

The conclusions reached, although obvious today, were revolutionary when these studies were published. We now take for granted that STEMI is generally caused by an acute thrombotic occlusion of an epicardial coronary artery and that timely recanalization of the occluded artery salvages jeopardized ischemic but still viable myocardium. Thirty years ago, such notions were thought by many to be heretical. Figure 1 summarizes the most relevant landmarks in the history of STEMI therapy (20).

The development of reperfusion as a therapy for limiting ischemic damage during STEMI is 1 of the greatest success stories in the treatment of human disease. Before this paradigm was established, early mortality was >20%; for example, the mortality rate in the control group of the GISSI-1 (Gruppo Italiano per lo Studio della Streptochinasi nell’Infarto Miocardio) trial was 18% (99). This figure has since declined to reach <5% in recent randomized clinical trials focusing on either pharmacological revascularization, mechanical revascularization, or both. Aside from reperfusion itself, the progressive reduction in the time between STEMI diagnosis and reperfusion has made an important contribution to this reduced mortality. Following the “time is muscle” principle, huge efforts have been made to ensure early reperfusion. It is now widely accepted that the shortening of door-to-ballon time (the time between first medical contact and mechanical reperfusion) results in greater myocardial salvage and better outcomes. A major multidisciplinary effort has resulted in a significant decline in door-to-balloon times over the last 10 years in all registries. However, despite these improvements, a large U.S. study of almost 100,000 STEMI patients found that in-hospital mortality has remained unchanged, indicating the need to target other components of the total ischemic time (3) and other factors that contribute to infarct size.

**REPERFUSION BY PRIMARY PERCUTANEOUS CORONARY INTERVENTION, THROMBOLYSIS, OR BOTH.** Reperfusion is the most effective therapy ever developed against ischemic damage during STEMI. Pharmacological, mechanical, and combined reperfusion techniques have been refined over the years, and their efficacy has been greatly advanced by the development of adjunctive therapies. The use of lytic agents in placebo-controlled trials was pivotal in the elucidation of the benefits of sustained reperfusion. The first large-scale trial to definitively show a significant reduction in mortality by reperfusion with intravenous administration of thrombolytic agents was the landmark GISSI-1 trial (99). This study was followed by other landmark trials in the field (100,101).

Another revolution in the search for improved therapies to reduce the ischemic damage associated
with STEMI was the use of mechanical reperfusion by percutaneous coronary intervention (PCI). PCI for STEMI (primary angioplasty) was first described as a rescue intervention in cases in which thrombolysis was unsuccessful. It was also implemented widely as adjunctive therapy to thrombolysis, and it was performed systematically to evaluate the coronary anatomy and residual stenosis or electively in cases of spontaneous or inducible angina days after successful thrombolysis. The use of primary angioplasty as an alternative to thrombolysis was first described in 1983 (102).

The many studies comparing these strategies leave no doubt that timely PCI by an experienced team is superior to in-hospital thrombolytic therapy (103). Furthermore, progress in stent therapy has markedly reduced the incidence of acute and late stent thrombosis (104) and refined the primary PCI strategy. Despite the clear advantages of PCI over thrombolysis in head-to-head comparisons, the clinical scenario is sometimes more complicated. Although no prospective studies have been performed to prove it, primary PCI may not exhibit a mortality advantage over immediate thrombolysis when performed after a delay of 120 min. In some patients (those who present early with a large AAR and a low bleeding risk), this maximum acceptable delay may be significantly shorter (105).
A slightly different approach that was recently developed for patients who cannot get timely PCI is to follow the classical thrombolytic therapy and delay the planned PCI until 3 to 24 h after lytic administration (this differs from the facilitated approach, in which PCI is performed as soon as the patient arrives at the PCI center). In the STREAM (Strategic Reperfusion Early After Myocardial Infarction) trial, pre-hospital administration of the thrombolytic tenecteplase (half-dose in the elderly) to ≈2,000 STEMI patients who could not get PCI within 1 h resulted in rates similar to standard primary PCI for the composite of death, shock, congestive heart failure, or reinfarction at 30 days (106). One-year mortality rates in the 2 groups were almost identical (107). This approach needs to be further explored in patients with long transport times to the PCI hospital.

The use of adjunctive antithrombotic agents with thrombolytics is predicated on the fact that the intensity and net extent of thrombolysis reflects a competition between lysis and ongoing thrombosis (108). Convincing evidence of the effectiveness of aspirin as an adjunctive agent was first acquired in the ISIS-2 (Second International Study of Infarct Survival) trial (109), in which the benefits of aspirin and streptokinase were additive. Aspirin has also been used as an adjunctive therapy in STEMI in patients undergoing reperfusion by primary PCI. Further clinical benefits are obtained by supplementing aspirin with new antiplatelet agents such as clopidogrel, ticagrelor, or prasugrel. Given the clear beneficial effect of optimal antiplatelet therapy in STEMI, it should be implemented in the testing of any cardioprotective strategy. Further information on reperfusion strategies and adjunctive pharmacological therapy can be found in dedicated review papers (20).

Hypothermia has been unequivocally shown to reduce the rate of progression of ischemic damage in animal models of STEMI (111), but clinical application of hypothermia has been extremely challenging due to the lack of a safe cooling procedure able to reduce temperature fast enough to affect ischemic damage (well before reperfusion). After several small studies, the CHILL-MI (Rapid Endovascular Catheter Core Cooling combined with cold saline as an Adjunct to Percutaneous Coronary Intervention For the Treatment of Acute Myocardial Infarction) trial recruited 120 STEMI patients scheduled for PCI and randomized them to standard care or a rapid cooling protocol (infusion of cold saline plus endovascular cooling device). Hypothermia did not reduce infarct size (normalized to AAR) as measured by CMR (112), despite achievement of the target temperature (<35°C) in >75% of patients at the time of reperfusion. Attaining significant reductions of ischemic damage (and infarct size) with hypothermia would require the target temperature to be reached long before reperfusion. With the significant reductions seen in door-to-balloon times, this seems very unlikely to be achieved with the available techniques.

**OTHER THERAPIES TO REDUCE REPERFUSION INJURY**

Although the history of therapies to reduce ischemic damage, mostly involving reperfusion therapy, is full of rapid successes, the development of therapies to reduce reperfusion injury has been disappointing. This disparity reflects the contrast between the straightforward problem presented by reducing ischemic injury (restoration of blood flow) and the more complex processes associated with reperfusion injury.

**THE LONG DEBATE IS COMING TO AN END: LETHAL REPERFUSION INJURY IS A REALITY.** The term “reperfusion injury” has been used for many decades to describe several events associated with reperfusion, some transitory (e.g., ventricular arrhythmias, myocardial stunning, and so on) and others permanent (e.g., death induced by reperfusion, known as lethal reperfusion injury). The existence of lethal reperfusion injury in STEMI has long been a matter of debate (24,113). Despite the convincing experimental evidence already outlined in this review, definitive clinical demonstration has been lacking. This is partly due to the gap between well-defined and controlled experimental models on the one hand and unclear human proofs-of-concept (i.e., “clinical models”) and trial designs on the other (114). Negative findings in infarct size reduction trials accumulated, giving currency to the idea, common among cardiologists and in
the pharmaceutical industry, that reperfusion injury was either a fantasy or at best a laboratory artifact. Studies in the early 1990s showed that reperfusion did not increase the transmural extent of infarction in canine hearts, suggesting an absence of reperfusion injury (115). The dog is a particular case, however, in which injury progresses slowly, and 90 to 180 min of coronary occlusion might not provoke significant reperfusion injury; it is known that ischemic and reperfusion injuries are linked, the degree of the former determining the extent of the latter. The idea of lethal reperfusion injury has since won progressive acceptance on the basis of evidence coming from clinical and basic science studies. Lethal reperfusion injury can be defined as a potentially preventable death of myocardium that was viable at the time of reperfusion and that is the consequence of events triggered or magnified by reperfusion. The fact that preventive maneuvers like post-conditioning limit infarct size without affecting ischemic injury is the best demonstration of the reality of lethal reperfusion injury.

**Nonpharmacological Interventions to Reduce Reperfusion Injury.** The first major breakthrough in nonpharmacological interventions was the definition of “ischemic pre-conditioning” by Murry et al. (116), who, in 1986, reported that brief cycles of ischemia and reperfusion performed before a prolonged coronary artery occlusion with reperfusion could dramatically reduce final infarct size in dogs. Given that total ischemic time was unaltered (even increased) by this intervention, this study suggested that there was more to limiting infarct size than a shorter duration of ischemia. This finding ushered in a paradigm shift, which stimulated a wave of research that has increased our understanding of the pathophysiology of I/R injury at the molecular level, thereby preparing for the identification of new targets for future innovative therapies. Many years later, the infarct-limiting effects of ischemic pre-conditioning were shown to be due to a large extent to a reduction in reperfusion injury (117), although it is also plausible that pre-conditioning can reduce ischemic damage. The unpredictability of coronary artery occlusion in STEMI patients means that ischemic pre-conditioning cannot be applied in this clinical setting, but it could have an important role in planned procedures like cardiac surgery (8,75). The second breakthrough was the description of “ischemic post-conditioning” by Zhao et al. (23) in 2003. They showed that brief episodes of ischemia and reperfusion performed immediately after reflow following prolonged ischemia could reduce final infarct size in dogs by 30% to 40% (23). This effect was even more surprising than pre-conditioning, because the intervention was applied after reflow; thus, it has no connection to the duration of ischemia or any associated event and must be related to the prevention of events occurring after reperfusion. With this simple experiment, this group demonstrated that lethal reperfusion injury is a reality (24), is quite significant in an experimental setting (30% to 40% of final infarct size), and that it is amenable to timely intervention.

With these breakthroughs, the time had come to test whether lethal reperfusion injury could be attenuated in STEMI patients. The first to do so were Staet al. (89), who demonstrated in 2005 that ischemic post-conditioning can reduce infarct size in STEMI. In this proof-of-concept trial, ischemic post-conditioning was applied within 1 min after reflow by inflating/déflating the angioplasty balloon (low-pressure, upstream of the stent) in 4 1-min cycles. This resulted in a 36% reduction of the area under the curve for creatine kinase release, a surrogate marker of infarct size. Most, not all, of the small trials performed have shown infarct size reduction in patients undergoing post-conditioning (8). However, the largest randomized clinical trial of post-conditioning in STEMI was neutral (118). The protocol for the POST (Effects of Postconditioning on Myocardial Reperfusion in Patients With ST-Segment Elevation Myocardial Infarction) trial allowed the discretionary use of thrombectomy, pre-dilation, and other maneuvers. The repeated balloon inflation-deflation at the site of the culprit lesion might have been responsible for excessive inadvertent thrombus microembolization, as suggested by the low rate of ST-segment resolution. And, although the protocol stipulated post-conditioning within 1 min of STEMI, the high frequency of thrombectomy (50%) likely delayed post-conditioning beyond the protective 1-min time-frame, and this might have diluted the benefits of this protective strategy. The fact that the largest trial was neutral calls for caution in the interpretation of the infarct-limiting effects of post-conditioning until new trials are completed. In addition, the details of the POST trial highlight that application of this strategy in a real-life scenario is more challenging than in the proof-of-concept trials, in which patient selection and protocol application is more controlled. In the DANAMI-3 (Danish Study of Optimal Acute Treatment of Patients With ST-elevation Myocardial Infarction-3) (NCT01435408), 2,000 STEMI patients will be allocated to 1 of 3 study interventions: conventional PCI (immediate stent implantation), post-conditioning (with stent implantation after the end of 4 30-s post-conditioning cycles), or deferred stenting (opening the culprit
artery with the wire only, followed by thrombectomy and/or low pressure balloon inflation and stenting after 48 h). The combined endpoint will be all-cause mortality or heart failure at 2 years.

Another form of myocardial conditioning well described in animal models is remote ischemic conditioning: conditioning performed in a distant organ (119). Remote ischemic conditioning is reviewed in detail elsewhere (120). For the purpose of this review, it is important to mention that remote ischemic preconditioning (4 5-min brachial cuff inflations applied during ongoing STEMI: i.e., during ambulance transfer to the PCI center and before PCI reperfusion) resulted in increased myocardial salvage compared with regular PCI (121), and this might translate into fewer long-term clinical events (122). The possibility should be confirmed in the ongoing CONDI-2 (Effect of RIC on Clinical Outcomes in STEMI Patients Undergoing pPCI) trial (NCT01857414).

PHARMACOLOGICAL INTERVENTIONS TO REDUCE REPERFUSION INJURY. During the past 10 years, many phase II clinical trials have been performed to find coadjuvant pharmacological interventions to ameliorate the myocardial damage associated with STEMI. We will describe here the most promising pharmacological strategies as well as examples from the long list of failures.

DRUGS WITH PROMISING RESULTS IN PILOT/PHASE II TRIALS

CYCLOSPORINE-A. Because local conditions (coronary anatomy, thrombus burden) can make it difficult to apply ischemic post-conditioning during PCI, pharmacological agents that trigger similar pathways to ischemic conditioning have been extensively investigated at the pre-clinical level and then translated into pilot clinical trials (123). Cyclosporine-A is the paradigm pharmacological post-conditioning agent. Cyclosporine-A acts by inhibiting the opening of the MPTP, an event also seen with post-conditioning. Piot et al. (90) randomized 58 patients to receive a single bolus of cyclosporine A or placebo immediately before PCI. Infarct size, measured by the area under the curve of creatine kinase, was significantly smaller in the cyclosporine-A group. The ongoing multicenter, randomized, placebo-controlled CIROCUS (Cyclosporine and Prognosis in Acute Myocardial Infarction [MI] Patients) trial (NCT01502774) recruited 975 anterior STEMI patients (Thrombolysis In Myocardial Infarction flow grade 0 to 1 left anterior descending occlusion) and randomized them to cyclosporine A or placebo. The combined primary endpoint (total mortality; hospitalization for heart failure; and LV remodeling [increase of LV end-diastolic volume >15%]) will be assessed at 1-year follow-up.

METOPROLOL. The effect of β-blocker agents were tested in many STEMI trials in the 1970s to 1980s with no definite conclusion on their cardioprotective effect. However, these trials were performed before reperfusion became established practice, and it is not surprising that β-blockers showed no consistent infarct limiting effect because, with no reperfusion, the chances of myocardium salvage are negligible. In the era of thrombolysis as the standard treatment for STEMI, the 1 randomized clinical trial performed showed neutral effects of intravenous (IV) atenolol on infarct size (110). Preclinical data from the pig model of infarction demonstrated that IV metoprolol very significantly reduces infarct size when administered before reperfusion (124,125). Contrary to the classical theory of reduced myocardial oxygen consumption, the mechanism responsible for this infarct-limiting effect is proposed to be related to a reduction in reperfusion injury due to the effect of metoprolol on circulating cells (neutrophils/platelets) rather than cardiomyocytes (126). This pre-clinical evidence led to the METOCARD-CNIC (Effect of Metoprolol in Cardioprotection During an Acute Myocardial Infarction) trial, in which 270 anterior STEMI patients undergoing PCI were randomized to early IV metoprolol or control before reperfusion. Infarcts, measured by CMR, were significantly smaller in the IV metoprolol group (86), and the effect was more pronounced in patients recruited during ambulance transfer to the PCI center (127). Six-month CMR follow-up of more than 200 patients showed that the IV metoprolol group had a significantly higher mean LVEF and had significantly less cases of severe LVEF depression (128).

The encouraging results from the METOCARD-CNIC trial appear to contradict findings from the much larger COMMIT (ClOpidogrel and Metoprolol in Myocardial Infarction Trial). In this mega trial, STEMI patients undergoing thrombolysis were randomized to early IV metoprolol or control before reperfusion. Infarcts, measured by CMR, were significantly smaller in the IV metoprolol group (86), and the effect was more pronounced in patients recruited during ambulance transfer to the PCI center (127). Six-month CMR follow-up of more than 200 patients showed that the IV metoprolol group had a significantly higher mean LVEF and had significantly less cases of severe LVEF depression (128).

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at a mean of >10 h after symptom onset (129). The amount of myocardial salvage that can be achieved after 10 h of coronary occlusion is residual at best, especially when thrombolysis is used as the reperfusion strategy (130). Second, the COMMIT trial included STEMI patients in Killip class III, who received the full regime of metoprolol administration; metoprolol resulted in relative mortality reductions of 5% and 2.5% in Killip class I and II patients, respectively, but increased mortality by 19% in Killip class III patients. Finally, metoprolol also increased mortality in patients with systolic blood pressure <120 mm Hg. These results reinforce the contraindications for IV β-blocker therapy in patients with overt heart failure or who are hemodynamically compromised, who have been systematically excluded from other β-blocker studies. In contrast with COMMIT, the METOCARD-CNIC trial recruited early presenters (<6 h from STEMI onset), and patients with Killip class ≥III were excluded. The patient population in the METOCARD-CNIC trial is thus more representative of the current standard of care for STEMI patients. The ongoing EARLY BAMI (Beta-blocker Administration before primary PCI in patients with ST-elevation Myocardial Infarction) trial (131) is testing the infarct-limiting effects of IV metoprolol in STEMI patients recruited during ambulance transfer to the PCI center, with a similar design to METOCARD-CNIC, except that it includes patients with infarcts in any location (METOCARD-CNIC recruited only patients with anterior STEMI) and extends the time window for recruitment to 12 h (compared with 6 h in METOCARD-CNIC). Finally, the hard endpoint-powered MOVE ON! (Impact of pre-reperfusion Metoprolol On clinical eVEnts after myocardial infarction) trial will definitively answer whether the amelioration of I/R injury exerted by early IV metoprolol (132) translates into a real clinical benefit. Given that not all β-blockers have the same intracellular effects (due to their disparate lipophilicity among other differences) and do not even share the same mechanistic effect (despite being considered β1 selective), it should not be assumed that all will have similar infarct-limiting effects.

**GLUCOSE MODULATORS.** The possible therapeutic use of glucose to protect cardiomyocytes from energy depletion during myocardial infarction was proposed several decades ago by Sodi Pallares et al. (133). Combined administration of glucose/insulin/potassium (GIK) during ongoing myocardial infarction has been tested in several trials with some encouraging results. The IMMEDIATE (Immediate Myocardial Metabolic Enhancement During Initial Assessment and Treatment in Emergency Care) trial recruited patients with suspected acute coronary syndrome and randomized them to GIK or placebo during transfer to the hospital. In the subgroup of patients presenting with STEMI, GIK significantly reduced CMR-evaluated infarct size (134). However, these promising results need to be confirmed in a prospective trial powered to detect differences in infarct size. Another approach has been the use of glucagon-like peptide-1 (GLP1) analogs. After promising preclinical results, Lonborg et al. (135) randomized 172 STEMI patients to receive IV injection of the GLP1 analog exenatide or placebo. Myocardial salvage on CMR was significantly higher in the exenatide group (135).

**ABCIXIMAB.** Glycoprotein IIb/IIIa inhibitors were developed for the reduction of thrombotic events due to their potent effect on platelets and platelet-leukocyte aggregates implicated in I/R injury. The INFUSE-AMI trial recruited 452 anterior STEMI patients undergoing PCI and performed an open-label, 2×2 factorial randomization to test the effect of abciximab and/or thrombectomy on infarct size, as evaluated by CMR. Thrombus aspiration had no effect on infarct size, but intracoronary administration of abciximab significantly reduced infarct size (136). Given that the current standard of care for STEMI patients includes the use of potent oral antiplatelet agents, glycoprotein IIb/IIIa inhibitors are left for a selected STEMI population.

**EXAMPLES FROM THE LONG LIST OF FAILED CLINICAL TRIALS FOR REDUCING REPERFUSION INJURY**

Many clinical trials have attempted and yet failed to demonstrate the ability of a given therapy to limit infarct size by reducing reperfusion injury. A common denominator in many of these failures is the weak or unclear benefit in the pre-clinical phase (137,138). A description of all of the negative trials in the field is beyond the scope of this review; here, we provide an update on the more recent negative trials.

Adenosine has been evaluated in several trials and did not show a clear infarct-limiting effect. In the most recent trials, adenosine was administered by intra-coronary injection at high doses, and myocardial salvage was evaluated by CMR. Adenosine had no effect on myocardial salvage or MVO (87,139). The effect of inhaled nitric oxide on infarct size in 250 STEMI patients undergoing PCI was very recently tested in the NOMI (Nitric Oxide for inhalation to reduce reperfusion injury in acute STEMI) trial (140). Nitric
Parameters (118), and biomarker release (90) have been observed in 229 STEMI patients undergoing PCI in the NIAMI (Intravenous sodium nitrite in acute ST-elevation myocardial infarction: a randomized controlled trial) trial (141). The authors did not find any difference in infarct size as evaluated by CMR, the primary endpoint of the study. The effect of intravenous TRO40303, a drug binding to an unclear molecular target in the outer mitochondrial membrane, was tested in the MITOCARE (Effect of intravenous TRO40303 as an adjunct to primary percutaneous coronary intervention for acute ST-elevation myocardial infarction) trial as an adjunct to primary percutaneous coronary intervention for acute STEMI. This study of 163 STEMI patients randomized to TRO40303 or placebo found no differences in infarct size either by biomarker release (primary endpoint) or CMR. Salvage index by CMR was also not different between groups (142). Several other trials have shown negative results in the past (reviewed in more detail by Kloner [9]).

CLINICAL TRIALS TARGETING ISCHEMIA/REPERFUSION INJURY: IMPORTANCE OF ENDPOINTS

Incorporation of new clinical evidence into clinical practice guidelines requires the demonstration of a clear clinical benefit (an effect on hard endpoints). This demonstration usually requires large phase III trials. Given the high costs associated with large trials, the natural next step after obtaining strong preclinical data is to perform a pilot (phase II) clinical trial. These small trials usually choose a primary endpoint accepted as a surrogate for hard clinical endpoints. Infarct size is the most intuitive parameter evidencing the cardioprotective effect of a given intervention and also correlating with clinical events. More importantly, it is well-defined in animal models, where it is recognized as the hallmark of cardioprotection. Although single photon emission computed tomography (121), electrocardiogram parameters (118), and biomarker release (90) have been widely used, CMR-measured infarct size is currently the most widely recommended technique for assessing infarct size in STEMI trials. LVEF is the classical surrogate functional parameter, because it has been clearly associated with long-term mortality and morbidity after STEMI (143). In addition to infarct size, other readouts of reperfusion injury or determinants of infarct size can be measured to more accurately evaluate protective interventions. For example, MVO is associated with poor clinical outcomes when evaluated by various techniques, mainly CMR (27,144). AAR has been used in recent trials to normalize infarct size or to depict myocardial salvage (% of AAR with no infarction). AAR can be estimated by angiographic means (BARI [Bypass Angioplasty Revascularization Investigation Myocardial Jeopardy Index]/APPROACH [Alberta Provincial Project for Outcome Assessment in Coronary Heart Disease] coronary angiographic scores or LV angiographic scores) or by T2-CMR. Although T2-CMR is increasingly used, its capacity to depict true AAR is debated (the pros and cons of the use of CMR to depict AAR are reviewed elsewhere [145,146]). Some studies have reported a prognostic value of myocardial salvage (147); however, this parameter has not been extensively validated. Myocardial salvage is a surrogate of infarct size used to control for variations in AAR between study groups. In addition, very recently, therapies that reduce infarct size, like post-conditioning (148) and remote conditioning (149), were shown to reduce CMR-evaluated AAR. Studies of remote ischemic conditioning and the GLP1 analog exenatide have shown increases in myocardial salvage, but no significant effect on infarct size or LVEF (121,135). These results should, moreover, be interpreted with caution, because the real surrogate markers of hard clinical endpoints were neutral. Trials using infarct size (a known surrogate of clinical events) as an endpoint are easier to interpret than trials using myocardial salvage (a surrogate of a surrogate of clinical events). The use of CMR to visualize post-infarction edema has recently been made more complex by the demonstration that the edematous reaction of the myocardium to ischemia/reperfusion is bimodal (150). Comprehensive serial CMR evaluation in pigs showed that there are 2 independent waves of edema after ischemia/reperfusion: an initial wave appearing abruptly upon reperfusion, and a deferred wave occurring a few days later (150). This bimodal post-ischemia/reperfusion edematous reaction highlights the need for caution in the use of CMR to visualize edema as a marker of ischemic memory.

Sample size calculation for clinical trials is based on the anticipated treatment effect on a principal outcome (a reduction or increase in the experimental arm vs. control). Normally, several additional secondary endpoints are prospectively evaluated. It is important to stress that when the primary endpoint of a trial is negative, all other findings are exploratory at best, and the secondary outcome, if relevant, should be tested in a dedicated trial. It is also important to highlight the risk of overinterpreting subgroup analyses. Some of the phase II trials described here as negative have “exploratory” analyses in which
subgroups (e.g., anterior STEMI or patients presenting early) show a positive result. These analyses are biased because the subgroup was not randomized, and thus, the results could be affected by potential unknown confounders. This is especially true when the overall population does not show a significant treatment effect. The interpretation of endpoints in clinical trials was recently reviewed by Pocock and Gersh (151).

Other relevant questions regarding the selection of populations for clinical trials are related to the better identification of potential responders according to the expected mechanism of the study intervention. The response to protective agents administered at reflow appears to vary according to the duration of ischemia, and some trials using pharmacological agents suggest that only patients with short duration of ischemia can benefit (112). This suggests that the dynamics of lethal reperfusion are influenced by the duration of the preceding ischemia and are probably not linear. In addition, indirect evidence suggests that age, sex, comorbidities (e.g., diabetes, hypertension, smoking), and cotreatments (statins, antiplatelet agents) may have an effect on infarct size and on protection by conditioning interventions (94,152).

THE FUTURE

Over the past decades, important progress has been made in the quality of phase II trials evaluating protective interventions against lethal reperfusion injury. Although these trials will always be a mandatory preliminary step, the challenge of the next decade is to set up larger phase III trials evaluating clinical outcomes to therapies targeting lethal reperfusion injury. As in other disciplines, we envision that the advances in the next decade will come from refining the therapies already available rather than identifying new drugs. The commitment of funding agencies, scientific societies, and industrial partners is needed to achieve this challenging goal.

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**KEY WORDS** Ischemia/reperfusion, myocardial infarction, STEMI, therapy
Remote Ischemic Conditioning

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ABSTRACT

In remote ischemic conditioning (RIC), brief, reversible episodes of ischemia with reperfusion in one vascular bed, tissue, or organ confer a global protective phenotype and render remote tissues and organs resistant to ischemia/reperfusion injury. The peripheral stimulus can be chemical, mechanical, or electrical and involves activation of peripheral sensory nerves. The signal transfer to the heart or other organs is through neuronal and humoral communications. Protection can be transferred, even across species, with plasma-derived dialysate and involves nitric oxide, stromal derived factor-1α, microribonucleic acid-144, but also other, not yet identified factors. Intracardiac signal transduction involves: adenosine, bradykinin, cytokines, and chemokines, which activate specific receptors; intracellular kinases; and mitochondrial function. RIC by repeated brief inflation/deflation of a blood pressure cuff protects against endothelial dysfunction and myocardial injury in percutaneous coronary interventions, coronary artery bypass grafting, and reperfused acute myocardial infarction. RIC is safe and effective, noninvasive, easily feasible, and inexpensive. (J Am Coll Cardiol 2015;65:177–95) © 2015 by the American College of Cardiology Foundation.

Remote ischemic conditioning (RIC) is the intriguing phenomenon whereby brief, reversible episodes of ischemia with reperfusion applied in one vascular bed, tissue, or organ confer global protection, rendering remote tissues and organs resistant to ischemia/reperfusion injury. Its discovery 2 decades ago in the heart (1) was not serendipitous, but evolved from a mathematical model developed by Whittaker and Przyklenk (2–4), in which brief episodes of pre-conditioning ischemia in one coronary bed were predicted to trigger activation, release, or transport of one or more unknown “protective factors” throughout the myocardium. To test this hypothesis, anesthetized dogs underwent 4 episodes of 5-min ischemia applied in the left circumflex coronary territory, followed by a 1-h sustained ischemic insult in the left anterior descending coronary artery bed. As anticipated, compared with control subjects that underwent left anterior descending occlusion alone, animals that received brief antecedent episodes of circumflex occlusion before sustained left anterior descending occlusion displayed a robust reduction of infarct size (1).
HISTORICAL BACKGROUND AND CONCEPT OF RIC

EVOLUTION OF THE PARADIGM. Although this first report of “intracardiac” RIC was provocative and met with considerable skepticism (4), the concept also engendered curiosity and raised the question: can the RIC paradigm be extrapolated to other remote triggers?

Spatial evolution: from intracardiac to interorgan RIC. During the past 2 decades, multiple variations on the theme of RIC have been investigated, encompassing both in vitro and in vivo models. Cardioprotection by collection and transfer of perfusate among isolated buffer-perfused hearts is a notable example (5-8). Specifically, coronary effluent released from donor rabbit hearts throughout a standard, conventional pre-conditioning stimulus (3 cycles of 5-min global ischemia with 10-min reperfusion) or a time-matched control period was collected, reoxygenated, warmed, and used as the perfusate for 2 cohorts of naïve, acceptor hearts. All 4 groups of hearts then underwent 40 min of sustained global ischemia. Infarct sizes were significantly smaller in both, donor hearts subjected to brief pre-conditioning ischemia and naïve acceptor hearts that received the effluent from pre-conditioned donors, versus donor and acceptor control subjects. There was no difference in the magnitude of the infarct-sparing effect seen in donor- and acceptor-pre-conditioning groups, implying that the efficacy of cardioprotection triggered by RIC was comparable to that achieved by conventional ischemic preconditioning (5). This general strategy, involving transfer of effluent or perfusate, has been refined to include collection of serum following brief pre-conditioning ischemia in vivo and its administration to either isolated hearts or cultured cells subjected to a sustained ischemic or hypoxic insult (9-11). This strategy also provided evidence of cross-species protection by RIC, including treatment of isolated buffer-perfused rabbit hearts with human serum (9,11).

It could be argued that intracardiac RIC or cardioprotection achieved by transfer of perfusate between hearts is a laboratory curiosity providing mechanistic insight, but of limited translational relevance. Accordingly, the observation of interorgan RIC was a pivotal pre-clinical advance (12). Initial evidence revealed that brief episodes of ischemia/reperfusion in kidney and mesentery rendered the heart resistant to infarction (12-15). Moreover, a number of studies documented RIC-induced attenuation of ischemia/reperfusion injury in brain, lungs, liver, kidney, intestine, skin, and other tissues (reviewed in Candilio et al. [16]). However, the first reported seminal extension of interorgan RIC in a clinically-relevant, large-animal (swine) model (17), which demonstrated that brief episodes of peripheral limb ischemia, achieved by simple tourniquet occlusion of one hindlimb, was sufficient to evoke a profound reduction in myocardial infarct size, accelerated subsequent implementation of phase II trials aimed at establishing efficacy in patients (17).

Conceptual evolution: from ischemic to non-ischemic triggers. In the aforementioned studies, intercardiac and interorgan RIC were (by definition) initiated by a brief ischemic stimulus. However, accumulating evidence from a spectrum of in vivo and in vitro models (some involving perfusate transfer among models) suggests that transient ischemia or interruption of blood flow is not a requisite trigger for remote protection. Multiple alternative triggers capable of recapitulating the infarct-sparing effect of RIC have been proposed, including peripheral noception (initiated by skin incisions made on the abdomen and termed “remote pre-conditioning of trauma”), direct peripheral nerve stimulation, and noninvasive transcutaneous nerve stimulation and electroacupuncture (18-23). Perhaps the most attractive, for its potential as a clinical cardioprotective strategy, is nontraumatic peripheral noception instigated by chemical stimulation of sensory C-fibers in the skin (18,21). A >70% reduction in infarct size was reported in mice treated with 0.1% capsaicin cream, applied topically to a 2 cm² area of skin along the abdominal midline 15 min before the onset of coronary artery occlusion, compared with untreated control subjects (18). In spite of its inherent appeal, this concept has not yet been translated to clinical investigation.

Temporal variants: remote pre-, per-, and post-conditioning. In all studies discussed thus far, the remote conditioning stimulus was administered prophylactically in the ~30- to 40-min period before the onset of sustained myocardial ischemia. However, pre-treatment is not a requirement for RIC-induced cardioprotection: reduction of infarct size has also been described with concurrent application of the remote ischemic stimulus during sustained coronary occlusion (remote ischemic per-conditioning) or at the time of reperfusion (remote ischemic post-conditioning) (24,25).

The first documentation of infarct size reduction with remote per-conditioning utilized brief renal ischemia/reperfusion as the trigger, applied during...
the final minutes of coronary artery occlusion (26). This approach provided proof-of-principle, but has obvious practical limitations as therapy. However, evidence from the swine model demonstrated a significant infarct-sparing effect of 4 5-min cycles of intermittent limb ischemia administered during a 40-min period of left anterior descending coronary occlusion (27), providing the rationale for the landmark clinical trial in which limb ischemia was applied during transport of patients with suspected acute myocardial infarction (AMI) to the hospital (28). Cardioprotection with remote ischemic post-conditioning was first demonstrated in swine (29) and subsequently corroborated in other models, including rabbit and rat (8,30). In each case, the protective stimulus was initiated immediately upon relief of the sustained myocardial ischemia.

The Pre-Clinical Consensus. A general consensus regarding RIC has emerged: with rare exceptions (31), there is consistent evidence among diverse models and species that brief ischemia/reperfusion applied in a remote tissue or organ confers cytoprotection against ischemia/reperfusion injury. When the heart is the target organ, the gold standard of RIC-induced protection is reduction of myocardial infarct size. However, remote ischemic pre-conditioning (RIPC) protects the myocardium, but also other parenchymal organs (16) and, notably, the vasculature. Endothelial dysfunction from ischemia/reperfusion can serve as a surrogate for studies on cardioprotection by RIC in healthy humans, but it is unclear whether extrapolation from preservation of peripheral vasomotion to cardioprotection is also true mechanistically (32).

Among the multiple variants of RIC, is any option superior for evoking cardioprotection? Interorgan (rather than intracardiac) conditioning, achieved via intermittent limb ischemia or, potentially, via non-traumatic peripheral nociception, is among the more appealing and practical strategies. Studies where peripheral limb ischemia is the RIC stimulus have mostly employed 3 or 4 episodes of 5-min arm and/or leg ischemia interspersed with 5-min reperfusion periods. However, these are empiric choices, the optimal algorithm has not been identified, and it has been postulated that “hyperconditioning” (i.e., an as-yet undefined, excessive number of conditioning episodes) may be deleterious (33,34). With regard to timing, outcomes of the limited number of head-to-head comparisons revealed no apparent difference in efficacy of RIPC, remote pre-conditioning, and post-conditioning (35,36). The paradigms of remote ischemic per-conditioning and post-conditioning may be particularly relevant, as they expand the potential scope for clinical translation of RIC.

SIGNAL TRANSDUCTION OF RIC

Neuronal Signal Transfer from the Remote Organ to the Heart. Signal transduction to the heart from the remote organ where the RIC protocol initiates protection appears to involve the somatomotor-sensory system, the spinal cord, and the autonomous nervous system (Central Illustration). The stimulus can originate not only from local ischemia/reperfusion injury in an organ other than the heart (e.g., mesentery [12,14,37] or limb [35,38–42]), but also from local surgical trauma (18–20,41); local activation of sensory fibers by capsaicin (18,21,35), bradykinin (14,20), or adenosine (39); and local electrical nerve stimulation (21,23). Accordingly, local anesthesia with lidocaine (18) or a sensory nerve blocker (21) and transection of the peripheral nerve (21,35,39,40) abrogated protection by RIPC, although femoral nerve transaction did not abrogate protection by limb RIPC in mice in one study (42).

The local release mechanism in response to a nociceptive stimulus involves protein kinase Cγ in rats (20) and is inhibited by a nitric oxide donor (39). Whereas the causal involvement of peripheral nociceptive sensory nerves is unequivocal, the nature and transfer to the heart of the released transmitter molecule through neuronal or humoral pathways remains ambiguous. A blood-derived dialysate was able to transfer protection to a recipient bioassay heart after local peripheral adenosine or capsaicin administration, peripheral nerve stimulation, or RIPC (21,39), supporting the notion of humoral transfer of a neuronally-released signal molecule. This is also suggested by studies in humans, where the dialysate from diabetic subjects after RIC provided protection only in the absence of diabetic neuropathy (11).

Abrogation of protection by RIPC with spinal cord transection at T7-T10 (18,40) or intrathecal spinal opioid receptor blockade with naloxone (41) and infarct size reduction by spinal cord stimulation by C8-T2 (43) favor a spinal reflex response. The efferent pathway appears to involve the autonomous nervous system. The ganglionic blocker, hexamethonium, abrogated protection by local bradykinin administration or RIPC in most (12,14,18), but not all (38) studies. Another ganglionic blocker, trimetaphan, also abrogated RIPC’s protection from ischemia/reperfusion-induced endothelial dysfunction in humans (44). Cardiac sympathetic nerves are involved in attenuation of the observed infarct size reduction upon spinal cord stimulation, and this effect is attenuated by the α1-blocker, prazosin, and the β-blocker, timolol (43). Another β-blocker, propranolol, also abrogated
protection by peripheral surgical trauma (18). Vagotomy (35,40) or atropine (40,45) abrogated the protection by limb pre-conditioning (35,40).

In conclusion, local injury during remote organ pre-conditioning activates nociceptive fibers, which release an unidentified molecule into the blood and/or signal through the spinal cord to activate both cardiac vagal and sympathetic efferents to release cardioprotective substances. Most of the previously discussed data originate from rodent models of RIC or from studies with transfer of dialysate to rodent hearts. However, neuronal involvement in protection by RIPC in humans undergoing coronary artery bypass grafting (CABG) or aortic valve surgery is suggested by its abrogation with propofol, but not isoflurane anesthesia (46–48).

**Humoral Signal Transfer from the Remote Organ to the Heart.** In early studies of local pre-conditioning, coronary effluent from a pre-conditioned heart induced cardioprotection in a naïve acceptor heart (5). The presence of a circulating cardioprotective factor after RIPC was first demonstrated in a porcine transplant model (49), where RIPC of the limb in an accepor pig provided potent cardioprotection to the subsequently transplanted and denervated donor heart. Subsequent studies confirmed the presence of a circulating element and further characterized the nature of the factor(s). In an
isolated rabbit heart model (9), plasma from remotely pre-conditioned animals was cardioprotective when perfused into an isolated naïve heart. The plasma dialysate using a 15-kDa membrane was similarly cardioprotective. When processed over a C18 column, the small hydrophobic molecule eluate provided potent cardioprotection, along with a protective kinase signature. Importantly, when the dialysate was given to isolated fresh cardiomyocytes (excluding neuronal influence), the resistance of cardiomyocytes to simulated ischemia/reperfusion injury mimicked that of a local pre-conditioning stimulus. Subsequent animal studies using such Langendorff bioassays confirmed that RIC induced by femoral nerve stimulation, transcutaneous peripheral nerve stimulation, capsaicin, and even electroacupuncture appear to work, at least in part, via release of cardioprotective factors into the blood (21–23,39).

Langendorff bioassays have also been used to test for the presence of circulating cardioprotective factors in human RIC. Depending on whether peripheral neuropathy was present, dialyzed plasma from diabetic patients subjected to RIC had differential responses, confirming interaction between the neural and humoral components of remote conditioning (11). Whereas plasma from diabetic patients without neuropathy was highly cardioprotective in naïve acceptor rabbit hearts, patients with peripheral neuropathy failed to provide cardioprotective plasma. Most recently, RIPC had no effect on exercise performance in heart failure patients (50). However, in the isolated mouse heart bioassay, plasma from heart failure patients was cardioprotective at baseline, but provided no additional cardioprotection after RIPC. When the results were stratified for the degree of baseline cardioprotection, those with low baseline cardioprotective activity showed significant improvement in their exercise function after clinical RIC (e.g., troponin, creatine kinase, or left ventricular ejection fraction), suggesting that the clinical effect of RIC is beyond that of nitrite alone.

Stromal-derived factor-1z is a small chemokine that fulfills the criteria for a putative circulating effector (9), and is cardioprotective via its interaction with its chemokine receptor 4 (54). Circulating plasma levels of stromal-derived factor-1z increased in rats subjected to RIPC by limb ischemia/reperfusion, and the cardioprotection of RIPC was partially abrogated by pre-treatment of the animals with a specific inhibitor (55). The lack of complete abrogation in this model suggests involvement of other factors.

Finally, a microribonucleic acid (microRNA) was recently shown to play a role in the pre-conditioning effect of transient limb ischemia/reperfusion. MicroRNA-144 levels were increased in mouse myocardium after RIPC and markedly reduced after ischemia/reperfusion injury. In subsequent experiments, the effect of RIC was completely abrogated by the use of a specific antagonim to microRNA-144. Conversely, intravenous microRNA-144 was cardioprotective, both acutely and 3 days after administration. Importantly, microRNA-144 levels were increased in the plasma of mice and humans subjected to limb RIC. Plasma carriage of microRNAs, to prevent digestion by circulating RNase, has been demonstrated within lipoprotein complexes in association with specific carrier proteins, such as argonaute, and in exosomes (56–59). Interestingly, the total number of exosomes in mouse plasma after RIC did not increase (60), although others observed increased numbers of exosomes following RIC in both rats and humans (61). However, the hairpin precursor of microRNA-144 in the exosome pellet increased 4-fold, and single-stranded microRNA-144 levels increased substantially in the plasma supernatant after RIC. Plasma microRNA-144 colocated with argonaute protein complexes, suggesting that this may be the plasma carriage mechanism after release of microRNA-144 precursor from the exosome. Although the exosome fraction was not tested for...
cardioprotective activity in that study, effluent from a pre-conditioned heart was able to protect a second heart unless microvesicles and exosomes were removed, demonstrating that protection depends upon their presence (62). In summary, more work is required to identify whether microRNA-144, other microRNAs, chemokines, and perhaps undiscovered circulating factors may act either alternately or in concert as the humoral signal transferring protection to the heart. Nitric oxide, stromal-derived factor-1z, and microRNA-144 are clearly humoral transfer signals, but they do not fully explain the RIC phenomenon.

**SIGNAL TRANSDUCTION OF RIC IN THE HEART.** The search for signaling molecules/mechanisms of RIC has largely focused on signals identified in local ischemic pre- and post-conditioning studies (63,64). Early studies using pharmacological antagonists identified the involvement of adenosine (13,26), bradykinin (14,19,65), opioids (37,66,67), epoxyeicosa trienoic acids (19), reactive oxygen species (66), and adenosine triphosphate (ATP)-dependent potassium channels (13,27), but could not dissect whether these molecules/mechanisms were involved in signal generation within the remote organ, transfer of the signal to the heart, cardioprotective signaling in the heart, or any combination of these steps. To attribute signaling to the heart, the signal must be demonstrated to localize in the myocardium or an antagonist must be given in the transfer fluid obtained after a RIC protocol in a donor organism, then administered to an isolated recipient and target heart. However, isolated bioassay hearts contain a number of different cellular compartments in addition to cardiomyocytes including innervation, vasculature, interstitial cells, and matrix with resident leukocytes/immune cells. Also, most signaling molecules/mechanisms have thus far only been determined in rodent hearts, and translation to larger mammals or humans cannot be taken for granted.

With these caveats in mind, there is solid evidence for a causal involvement of the ligands adenosine (10,68), bradykinin (18), interleukin-10 (in delayed RIPC) (69) and stromal-derived factor-1z (55) in the heart. Adenosine acts on its A1 receptor, which, in turn, interacts with δ and κ opioid receptors (68); bradykinin acts on its B2 receptor (18); and stromal-derived factor-1z acts on chemokine receptor 4 (55). Adenosine receptor activation results in improved mitochondrial function, as evidenced by better respiration and reduced formation of reactive oxygen species (10). Bradykinin B2 receptor activation results in protein kinase Cε activation (18). The action of interleukin 10 results in increased phosphorylation of protein kinase B (Akt) and endothelial nitric oxide synthase (69). RIC consistently results in activation of the reperfusion injury salvage kinase (RISK) pathway, that is, activation of phosphatidylinositol-4,5-bisphosphate 3-kinase (70), Akt (8,69-72), extracellular-regulated kinase 1/2 (71), and glycogen synthase kinase 3β (73). RISK activation was also confirmed by abrogation of infarct size reduction with the respective pharmacological antagonists (8,70,71) and was not only seen in rodent, but also in pig hearts (70), in which RISK activation was previously not found important for protection by ischemic post-conditioning (74). However, the study with RISK activation by remote ischemic pre- and per-conditioning in pigs was confounded by the ambiguous finding that an adenosine antagonist abrogated RISK activation, rather than protection (70). RIC also consistently (18,38,43,65) results in activation of protein kinase C, a key molecule in cardioprotection (75) with a somewhat ambiguous role (76,77); in rodent hearts, protein kinase Cε is classically activated and shifted from the cytosolic to the particulate fraction (18,65). The role of hypoxia-inducible factor (HIF)-1z in RIC is controversial; in 1 study, infarct size reduction by limb RIPC was abrogated in heterozygous knockout mice (72), but in another study, HIF-1z expression was increased by limb RIPC in wild-type mice, but was not a prerequisite for protection (73). HIF-1z protein expression is also increased in right atrial tissue of patients undergoing cardiac surgery under cardiopulmonary bypass with RIPC, but its causal involvement in the observed attenuation of troponin T release remains unclear (78). Late RIPC in rats increased heme oxygenase-1 protein expression, and its inhibition by zinc protoporphyrin abrogated protection (79). As in local ischemic pre-conditioning (80), limb RIPC in rats not only reduced infarct size, but also preserved connexin-43 phosphorylation and localization at intercalated disks (81); the role of mitochondrial connexin-43 in RIC has not been addressed.

An unbiased (mass spectrometry) proteomic search for phosphorylated proteins revealed that limb RIC increased expression of several phosphoproteins related to the sarcomeric Z-disc (82). A comprehensive immunoblotting approach for established cardioprotective proteins in right ventricular tissue of children undergoing repair of Fallot’s tetralogy revealed no differences in their phosphorylated forms without or with RIPC (83). In left ventricular biopsies from adult patients undergoing CABG, tyrosine-phosphorylated signal transducer and activator of transcription 5 was the only protein among more than 30 established cardioprotective proteins that
was increased by RIPC (84). Autophagy appears to have no role in human RIPC (85).

Mitochondria are clearly involved in cardioprotection by RIC. Human plasma from healthy volunteers undergoing an RIC protocol had increased nitrite concentration and increased the concentration of myocardial nitrite when transferred to an isolated mouse bioassay heart. Myocardial nitrite was converted to bioactive nitric oxide by myoglobin and reduced infarct size. In parallel mouse experiments, the same nitrite-nitric oxide pathway was activated by RIPC, induced S-nitrosation of mitochondrial proteins, and reduced complex I respiration and reactive oxygen species formation (42). In rabbits with limb RIPC, blockade of the mitochondrial aldehyde dehydrogenase-2 by cyanamide abrogated protection; in parallel experiments in humans with a functionally inactive enzyme polymorphism, endothelial protection by RIPC was eliminated, supporting the concept that mitochondrial function is essential in RIC (86). Better preservation of mitochondrial respiration was also seen in right atrial tissue of patients undergoing CABG with RIPC, who also had lower incidence of post-operative atrial fibrillation (87). Apart from mitochondrial function, RIPC increases myocardial glycolytic flux in adult, but not in neonatal rabbit hearts along with reduced infarct size in adult, but not in neonatal hearts (88). In isolated hearts from rats that underwent a RIPC protocol, myocardial microRNA-1, microRNA-2, heat shock protein-70, and programmed cell death protein expression were decreased (89). In right atrial tissue of patients undergoing CABG with RIPC, microRNA-388-3p expression was increased (87). The biological meaning of these changes in microRNA expression is unclear.

Apparently, the intracardiac signal transduction of RIC largely resembles that of local ischemic pre- and post-conditioning, with significant involvement of nitric oxide, protein kinase C, the RISK pathway, and mitochondrial function. The data on myocardial signal transduction of RIC have not yet been integrated into a more complex and comprehensive scheme. Surprisingly, the role in RIC of the survival activating factor enhancement pathway, including signal transducer and activator of transcription 3, has not been addressed.

**CLINICAL EVIDENCE FOR RIC**

**EFFECTS OF RIC ON THE HEART: ELECTIVE ISCHEMIA/REPERFUSION.** Patients undergoing elective CABG and percutaneous coronary intervention (PCI) change as the demographics of the general population alter. The percentage of patients age ≥75 years at the time of operation increased from 17% in 1999 to 29% in 2005 (90). Operated patients had more comorbidities, with increased rates of hypertension (from 43.7% in 1999 to 68.9% in 2007) and obesity (from 13% to 17.5% in the same period) and worse functional and cardiac status (reduced ejection fraction, hemodynamic instability, and shock) (90). Improvement in anesthetics and surgical and perioperative treatments allows surgeons to accept patients for operation who, only a few years ago, would have been refused. Left ventricular ejection fraction <30% remains the most important determinant of outcome after isolated CABG (91). In elective CABG and PCI, adverse intermediate and long-term outcomes relate to periprocedural myocardial injury, including reduction of left ventricular ejection fraction; hence, the importance of cardioprotection beyond cardioplegia and off-pump surgery. Pre-conditioning by intermittent cross-clamping of the ascending aorta is invasive and has recently been comprehensively reviewed (64,92).

The first (very small) clinical study evaluating the effect of RIPC on creatine kinase-myocardial band release in CABG patients was negative (93). Translation of RIPC’s protective potential to forearm endothelium-dependent vasomotion (17) initiated exploration of the cardioprotective potential of this approach using biomarkers as an endpoint in cardiac surgery, such as pediatric cardiac surgery, CABG, and combined CABG and valvular surgery. Most studies, including one small pilot study of high-risk patients (93-102), demonstrated cardioprotective potential (46,84,87,103-116) (Table 1), with similar findings for elective PCI (117-125) (Table 2). Many studies only included a few patients. Type 2 error might explain the discrepant results and confounding factors, including age, comedication, anesthesia, morbidity, and risk factors, may also have influenced the efficacy of RIC (126). Concomitant therapy with beta-blockers (127,128) and statins (129) is cardioprotective, as is an anesthetic regimen using propofol or volatile anesthetics (46,48,128), and may interfere with the cardioprotective effect of RIC. The interference of propofol, which is cardioprotective per se, with further protection by RIC contrasts with the inherent cardioprotective effect of isoflurane, which does not interfere with RIPC (46,48), suggesting a specific interaction of propofol with neuronal transfer of the protective RIPC signal. Although in experimental studies, diabetes mellitus attenuated the effect of local ischemic pre-conditioning (130), the degree of cardioprotection may depend on stimulus intensity (131) and diabetes duration (132), and the
<table>
<thead>
<tr>
<th>First Author, Year (Ref. #)</th>
<th>Patients, n (Control/RIC)</th>
<th>Type of Surgery</th>
<th>RIC Regimen</th>
<th>Endpoint</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Günaydin et al., 2000 (93)</td>
<td>4/4 CABG</td>
<td>Upper limb</td>
<td>2 cycles I/R (3/2 min)</td>
<td>CK (Sampled via coronary perfusion catheter 5 min after declamping)</td>
<td>No effect</td>
</tr>
<tr>
<td>Cheung et al., 2006 (103)</td>
<td>20/17 Pediatric cardiac surgery</td>
<td>Upper limb</td>
<td>4 cycles I/R (5/5 min)</td>
<td>TnT (AUC 24 h after surgery)</td>
<td>Reduced TnT, reduced post-operative inotropic need, reduced lung function</td>
</tr>
<tr>
<td>Hausenloy et al., 2007 (104)</td>
<td>30/27 CABG</td>
<td>Upper limb</td>
<td>3 cycles I/R (5/5 min)</td>
<td>TnT (AUC 72 h after surgery)</td>
<td>43% reduction of TnT</td>
</tr>
<tr>
<td>Venugopal et al., 2009 (105)</td>
<td>22/23 CABG (cold blood cardioplegia)</td>
<td>Upper limb</td>
<td>4 cycles I/R (5/5 min)</td>
<td>TnI (plasma levels 4, 8, 12, 24, 48, and 72 h after surgery)</td>
<td>Reduced TnI with 3 cycles I/R (5/5 min) but not with 3 cycles I/R (10/10 min)</td>
</tr>
<tr>
<td>Hong et al., 2009 (106)</td>
<td>22/23 CABG (off-pump)</td>
<td>Upper limb</td>
<td>3 cycles I/R (5/5 min)</td>
<td>TnI (AUC 72 h after surgery)</td>
<td>No effect</td>
</tr>
<tr>
<td>Venugopal et al., 2009 (105)</td>
<td>22/23 CABG (cold blood cardioplegia)</td>
<td>Upper limb</td>
<td>4 cycles I/R (5/5 min)</td>
<td>TnI (plasma levels 4, 8, 12, 24, 48, and 72 h after surgery)</td>
<td>Reduced TnI with 3 cycles I/R (5/5 min) but not with 3 cycles I/R (10/10 min)</td>
</tr>
<tr>
<td>Li, 2010 (108)</td>
<td>27/26 Valve replacement</td>
<td>Lower limb</td>
<td>3 cycles I/R (4/4 min)</td>
<td>TnI (AUC 72 h after surgery)</td>
<td>No effect</td>
</tr>
<tr>
<td>Zhou (2010) (109)</td>
<td>30/30 Pediatric cardiac surgery</td>
<td>Upper limb</td>
<td>2 cycles I/R (5/5 min) 24 and 1 h prior to operation</td>
<td>CK-MB (plasma levels 2, 4, 12, and 24 h after surgery)</td>
<td>Reduced CK-MB and inflammatory biomarkers, improved post-operative lung function</td>
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<tr>
<td>Wagner et al., 2010 (110)</td>
<td>34/32 CABG (cold crystalloid cardioplegia)</td>
<td>Upper limb</td>
<td>3 cycles I/R (5/5 min) 18 h prior to operation</td>
<td>TnI (AUC 24 h after surgery)</td>
<td>Reduced TnI</td>
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<tr>
<td>Ali et al., 2010 (111)</td>
<td>50/50 CABG</td>
<td>Upper limb</td>
<td>3 cycles I/R (5/5 min)</td>
<td>CK-MB (plasma levels 8, 16, 24, and 48 h after surgery)</td>
<td>Reduced CK-MB</td>
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<td>Karuppusamy et al., 2011 (97)</td>
<td>27/27 CABG</td>
<td>Upper limb</td>
<td>3 cycles I/R (5/5 min)</td>
<td>TnI (AUC 48 h after surgery)</td>
<td>No effect</td>
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<tr>
<td>Wu et al., 2011 (112)</td>
<td>25/25 Mitral valve replacement</td>
<td>Upper limb</td>
<td>3 cycles I/R (5/5 min) and 3 cycles I/R (5/5 min) + 2 cycles I/R (10/10 min)</td>
<td>TnI (plasma levels 4, 8, 12, 24, 48, and 72 h after surgery)</td>
<td>Reduced TnI with 3 cycles I/R (5/5 min) but not with 3 cycles I/R (10/10 min)</td>
</tr>
<tr>
<td>Kottenberg et al., 2012 (46)</td>
<td>19/20 Propofol vs. isoflurane anesthesia</td>
<td>Upper limb</td>
<td>3 cycles I/R (5/5 min)</td>
<td>TnI (AUC 72 h after surgery)</td>
<td>Reduced TnI with isoflurane, but not with propofol anesthesia</td>
</tr>
<tr>
<td>Young et al., 2012 (94)</td>
<td>48/48 Cardiac surgery (high-risk CABG and valve surgery)</td>
<td>Upper limb</td>
<td>3 cycles I/R (5/5 min)</td>
<td>TnT (plasma levels 6 and 12 h after surgery)</td>
<td>No effect</td>
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<tr>
<td>Heusch et al., 2012 (84)</td>
<td>12/12 CABG</td>
<td>Upper limb</td>
<td>3 cycles I/R (5/5 min)</td>
<td>TnI (AUC 72 h after operation)</td>
<td>Reduced TnI</td>
</tr>
<tr>
<td>Lee et al., 2012 (98)</td>
<td>28/27 Pulmonary hypertensive infants receiving ventricular septal defect repair</td>
<td>Lower limb</td>
<td>4 cycles I/R (5/5 min)</td>
<td>TnI (AUC 24 h after surgery)</td>
<td>No effect</td>
</tr>
<tr>
<td>Pavione et al., 2012 (99)</td>
<td>10/12 Pediatric cardiac surgery</td>
<td>Lower limb</td>
<td>4 cycles I/R (5/5 min)</td>
<td>TnI (plasma levels 4, 12, 24, and 48 h after surgery)</td>
<td>No effect</td>
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<tr>
<td>Lucchini et al., 2012 (100)</td>
<td>28/27 CABG Opioids and propofol for induction and isoflurane for maintenance anesthesia</td>
<td>Lower limb</td>
<td>4 cycles I/R (5/5 min)</td>
<td>TnT (plasma levels 24, 48, and 72 h after surgery)</td>
<td>No effect</td>
</tr>
<tr>
<td>Xie et al., 2012 (113)</td>
<td>35/38 Valve surgery</td>
<td>Upper limb</td>
<td>3 cycles I/R (5/5 min)</td>
<td>TnI (plasma levels 6, 12, 24, 48, and 72 h after surgery)</td>
<td>Reduced TnI</td>
</tr>
<tr>
<td>Thielmann et al., 2013 (114)</td>
<td>167/162 CABG (cold crystalloid cardioplegia and cardiopulmonary bypass)</td>
<td>Upper limb</td>
<td>3 cycles I/R (5/5 min)</td>
<td>TnI (AUC 72 h after surgery)</td>
<td>27% reduction of TnI, reduced all-cause mortality</td>
</tr>
<tr>
<td>Ahmad et al., 2014 (101)</td>
<td>32/35 CABG On-pump</td>
<td>?</td>
<td>?</td>
<td>CK-MB (plasma levels 1, 12, 24, and 48 h after surgery)</td>
<td>No effect</td>
</tr>
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</table>
attenuation of protection by RIC seems minor in the clinical setting (133,134). Importantly, recent larger studies have not only relied on surrogate markers of cardioprotection, but also included long-term clinical outcomes and demonstrated a reduction of major cardiovascular events by RIPC up to 4 years after CABG (114) and up to 6 years after elective PCI (119). A recent study randomized 1,280 patients scheduled for elective cardiac surgery to control or RIPC and remote post-conditioning (95). RIC was given as 2 cycles of 5-min ischemia and 5 min of reperfusion on the upper arm before cardiopulmonary bypass or coronary anastomoses in those who had beating heart surgery, and was repeated in the same sequence immediately after bypass. Although the cardioprotective effect was not documented by a reduction of post-operative biomarker release, RIC did not reduce the primary endpoint, a composite of major adverse outcomes including death, myocardial infarction, arrhythmia, stroke, coma, renal damage, respiratory failure, gastrointestinal complications, and multiorgan failure, suggesting that this endpoint may have been too broad. Although RIC is thought to have systemic protective effects on various distal organs, the results are debatable because the composite endpoint differs from other studies yielding beneficial results. Moreover, the heterogeneity of the patient group, including CABG, cardiac valve surgery, and their combination, as well as ascending or transverse aortic surgery and congenital heart defect repair, may have introduced bias.

Recent meta-analyses demonstrated that RIPC reduces biomarkers in patients undergoing CABG (92,135). The 2 follow-up studies with clinical outcomes were single-center trials, not powered to demonstrate definitive answers about clinical outcome (114,120). The consistency of the beneficial clinical outcome in the studies adds credibility to a clinically-relevant benefit of RIC in relation to CABG and elective PCI. However, larger multicenter studies are still required to clarify the extent to which these findings translate into clinical benefit. Future studies should include high-risk patients, who might benefit most from protection by RIC, and preferably avoid propofol in their anesthetic regimen when specific cardioprotective effects are addressed.

**EFFECTS OF RIC ON THE HEART: AMI.** Although the incidence of AMI is declining in the Western World (136,137), ischemic heart disease is still the leading cause of death worldwide (138). Improvements in treatment have changed the epidemiology after AMI, with markedly improved 30-day survival, but have less favorably influenced long-term survival (136,139). Consistent with this, due to remodeling and heart failure (140), nonfatal ischemic heart disease has increased more than ischemic heart disease deaths since 1990 (138). The declining incidence of heart failure after AMI has not reached the magnitude that we might have expected from clinical trial data (139) and the prevalence is increasing (138). Consequently, one of the potentially most important applications of RIC may be in patients with AMI (28,141-147) (Table 3).

### Table 1 Continued

<table>
<thead>
<tr>
<th>First Author, Year (Ref. #)</th>
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</tr>
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<tbody>
<tr>
<td>Hong et al., 2014 (95)</td>
<td>636/644</td>
<td>Cardiac surgery (cardiac valve surgery, CABG, combined valve and CABG surgery, ascending aorta and aortic arch surgery, and congenital heart defect repair)</td>
<td>Upper limb 4 cycles I/R (5/5 min) - 2 cycles before and 2 cycles after cardiopulmonary bypass</td>
<td>Clinical outcome (composite of: death, myocardial infarction, arrhythmia, stroke, coma, renal damage, respiratory failure, gastrointestinal complications, and multiorgan failure)</td>
<td>No effect</td>
</tr>
<tr>
<td>Slagsvold et al., 2014 (87)</td>
<td>30/30</td>
<td>CABG</td>
<td>Upper limb 3 cycles I/R (5/5 min)</td>
<td>Mitochondrial oxidation</td>
<td>Improved mitochondrial respiration</td>
</tr>
<tr>
<td>McCrindle et al., 2014 (102)</td>
<td>151/148</td>
<td>Pediatric cardiac surgery</td>
<td>Lower limb 4 cycles I/R (5/5 min)</td>
<td>Duration of post-operative hospital stay</td>
<td>No effect</td>
</tr>
<tr>
<td>Holmberg et al., 2014 (116)</td>
<td>23/23</td>
<td>Cardiac surgery (CABG, valve surgery, ascending aorta, myxoma)</td>
<td>Upper limb 3 cycles I/R (5/5 min)</td>
<td>TnT (AUC 72 h after surgery)</td>
<td>25% reduction of TnT, NS</td>
</tr>
<tr>
<td>Candilio et al., 2014 (115)</td>
<td>90/90</td>
<td>Cardiac surgery (CABG and/or valve surgery)</td>
<td>Upper and lower limb 2 cycles I/R (5/5 min)</td>
<td>Perioperative myocardial infarction (AUC 52 h after surgery)</td>
<td>26% reduction of TnT Reduction of incidence of AF, renal failure, stay at ICU</td>
</tr>
</tbody>
</table>

AF = atrial fibrillation; AUC = area under curve; CABG = coronary artery bypass grafting; CK = creatine kinase; CK-MB = creatine kinase-myocardial band; I = ischemia; ICU = intensive care unit; NS = not significant; R = reperfusion; RIC = remote ischemic conditioning; STAT5 = signal transducer and activator of transcription 5; Tn = troponin.
Most clinical studies on infarct size after coronary revascularization have used indirect estimates of tissue damage, such as release of biomarkers and resolution of ST-segment elevation (148,149). Direct visualization of the area-at-risk and final infarct size to calculate the salvage index (proportion of salvaged area-at-risk) can be achieved by myocardial perfusion imaging using 99technetium-sestamibi single-photon emission computerized tomography (150) or cardiac magnetic resonance (CMR) imaging (151–153). CMR quantification of the area-at-risk poses challenges, because the optimal protocol to quantify edema, thought to represent area-at-risk, is not defined (154–156) and because any cardioprotective intervention that reduces final infarct size also may reduce edema (157,158), potentially underestimating salvage.

The first proof-of-concept study demonstrating that RIC can increase myocardial salvage investigated 333 patients undergoing primary PCI for STEMI, of whom 132 had available imaging data (28).

### TABLE 3 Clinical Studies of RIC in AMI

<table>
<thead>
<tr>
<th>First Author, Year (Ref. #)</th>
<th>Patients, n (Control/RIC)</th>
<th>RIC Regimen</th>
<th>Endpoint</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Battler et al., 2010 (28)</td>
<td>69/73</td>
<td>Upper limb 4 cycles I/R (5/5 min)</td>
<td>Salvage index (SPECT)</td>
<td>20% increase in salvage index</td>
</tr>
<tr>
<td>Munk et al., 2010 (141)</td>
<td>110/108</td>
<td>Upper limb 4 cycles I/R (5/5 min)</td>
<td>LVEF at 30 days</td>
<td>5% increase in LVEF in anterior infarcts</td>
</tr>
<tr>
<td>Rentoulas et al., 2010 (142)</td>
<td>30/33</td>
<td>Upper limb 3 cycles I/R (5/5 min)</td>
<td>ST-segment resolution</td>
<td>20% increase in proportion of patients achieving full ST-segment resolution</td>
</tr>
<tr>
<td>Crimi et al., 2013 (143)</td>
<td>50/50</td>
<td>Lower limb 3 cycles I/R (5/5 min)</td>
<td>CK-MB (AUC 72 h after PCI)</td>
<td>20% reduction of CK-MB release</td>
</tr>
<tr>
<td>Prunier et al., 2014 (144)</td>
<td>17/18</td>
<td>Upper limb 4 cycles I/R (5/5 min)</td>
<td>CK-MB (AUC 72 h after PCI)</td>
<td>31% reduction of CK-MB release</td>
</tr>
<tr>
<td>Sloth et al., 2014 (145)</td>
<td>167/166</td>
<td>Upper limb 4 cycles I/R (5/5 min)</td>
<td>MACCE at 4 years</td>
<td>12% reduction in MACCE</td>
</tr>
<tr>
<td>Hasenfus et al., 2014 (147)</td>
<td>260/260</td>
<td>Upper limb 4 cycles I/R (5/5 min)</td>
<td>TnT (AUC 24 h after PCI)</td>
<td>17% reduction of TnT release</td>
</tr>
<tr>
<td>White et al., 2014 (146)</td>
<td>40/43</td>
<td>Upper limb 4 cycles I/R (5/5 min)</td>
<td>CMR</td>
<td>27% reduction of infarct size</td>
</tr>
</tbody>
</table>

LVEF = left ventricular ejection fraction; SPECT = single photon emission computerized tomography; other abbreviations as in Tables 1 and 2.
A simultaneous study demonstrated that RIC increases the number of patients achieving complete ST-segment resolution and found a statistically borderline reduction of troponin-T release (142). In the former study, RIC was applied as 4 cycles of 5-min upper arm ischemia and 5-min reperfusion and was initiated in the ambulance during transportation to primary PCI. RIC increased salvage by 36% and tended to reduce final infarct size. In patients with anterior infarcts and patients with occluded culprit artery (Thrombolysis In Myocardial Infarction [TIMI] grade 0 to 1) on admission, infarct size reduction, as measured by single-photon emission computed tomography, was 44% and 31%, respectively, indicating that patients at highest risk benefit more from RIC as an adjunctive therapy to primary PCI. The findings translated into an increment of left ventricular ejection fraction in anterior infarcts (143). Although not powered to evaluate clinical outcome, a follow-up study of the total cohort showed that the beneficial effect of RIC translated into a reduction of major cardiovascular events up to 4 years after the index event (145). In a recent study, 4 cycles of 5-min cuff inflation/5-min deflation on the upper arm reduced myocardial edema and reduced infarct size, reflected by troponin release and CMR (146).

The first window of protection lasts for 2 to 3 h and onset appears to be instant, as RIC initiated immediately prior to revascularization also reduces infarct size in STEMI patients (144). In some protocol algorithms, remote pre-conditioning combined with local post-conditioning reduced infarct size in rats (71). This additive effect was not seen for remote pre-conditioning combined with local post-conditioning in the clinical setting of patients with reperfused AMI (144).

In a recent randomized study of 100 patients, remote post-conditioning also reduced infarct size, as assessed by the area under the curve of creatine kinase-myocardial band release (143). Infarct size was consistently reduced, as reflected by delayed gadolinium-enhancement volume on CMR and ST-segment resolution >50% in twice as many patients in the treatment than in the control group. After 1-year follow-up, 1 patient in the control group (refractory heart failure) and none in the post-conditioning group had died, and cardiovascular events were reduced in the treatment group. The beneficial effect was obtained by 3 cycles of 5-min/5-min blood pressure cuff inflation/deflation of the lower limb initiated at the time of reperfusion by balloon inflation or thrombectomy. Although a recent clinical study suggested that 1 occlusion cycle induces protection during elective PCI (125), experimental data from mice indicate that cardioprotective efficacy is determined by the number and duration of inflations (34).

Present reperfusion therapy is effective in the majority of patients undergoing primary PCI. It may be difficult to demonstrate additional clinical benefit from further intervention because this would require the demonstration of further reduction in small myocardial infarcts and its translation into a clinical benefit. A subgroup of patients undergoing not only primary PCI, but also elective PCI and CABG, develops serious complications, including extensive myocardial injury, which is most frequently vascular in origin. Although pre-clinical human data indicate that RIC may modify thrombogenesis (159,160) and yield cardioprotection beyond an unequivocal reduction of infarct size (e.g. by anti-inflammatory mechanisms) (161), the clinical implications are yet unknown. However, some patients, predominantly those with large anterior infarcts, develop heart failure due to myocardial injury and subsequent left ventricular remodeling several months or years after the infarct, despite optimal medical treatment according to guidelines (140). Because RIC reduces final tissue necrosis, improved clinical outcome must be assessed by reduced post-infarction left ventricular dysfunction and heart failure, combined with mortality reduction (162). To achieve widespread clinical acceptance of RIC, focus should be kept on patients at risk of extensive myocardial injury and global tissue damage. Its potential clinical utility is far from fully explored.

An emerging concept, known as chronic conditioning, is the daily use of RIC for a period of weeks. In rats, RIC administration daily for the first 28 days after myocardial infarction had a dose-dependent effect on cardiac remodeling, heart failure, and even death rate in the absence of a significant reduction of infarct size (163). This effect demonstrates benefits beyond modification of acute ischemic effects. However, in a recent pilot study, this did not immediately translate into improved exercise capacity in heart failure patients (50).

**CONFOUNDING FACTORS IN RIC**

No recognized effective therapeutic intervention for protecting the myocardium against the detrimental effects of ischemia-reperfusion injury presently exists. A major reason for this unfortunate situation is the inability to take the relevance of confounding factors present in the majority of basic and clinical studies into account; RIC studies are no different in this regard (126).
INFARCT LOCATION/PATIENT SELECTION. Only a quarter of all STEMI patients have infarcts of sufficient size to benefit from adjunctive therapy (164). Patients presenting with right and/or circumflex coronary artery occlusion, where the infarct is relatively small, do not benefit as much from cardioprotective therapy as those presenting with proximal left anterior descending coronary artery occlusion, where the infarct is significantly larger (28,165). “All-comer” trials will lead to the recruitment of far more patients with small infarcts and little additional myocardial salvage, which may actually dilute the positive effect elicited by any novel protective strategy. Alternatively, limiting recruitment to patients with large anterior infarcts is more challenging because they are the most ill (166); however, the benefit of proof-of-concept trials is that demonstration of a significant difference between treatment and placebo requires recruitment of fewer patients (167).

CONTROL OF TIMI FLOW PRIOR TO RIC. Some patients presenting with an AMI have already undergone spontaneous reperfusion prior to interventional reperfusion and are not likely to benefit from a therapy designed to protect against reperfusion injury (168). Therefore, only those patients with TIMI scores <1 should be included in such studies (28).

IMPORTANCE OF CORONARY COLLATERALS. The coronary collateral circulation’s ability to influence the size of an evolving myocardial infarction cannot be underestimated. In STEMI patients, substantial collateralization reduces the sizes of the area at risk and the evolving infarct. The extent of collateralization will thus negatively influence the ability to demonstrate an effect of any novel cardioprotective strategy. Patients with visible collaterals (Rentrop grade ≥1) should, therefore, be excluded (169).

DURATION OF CHEST PAIN AND TIMING OF INTERVENTION. Patients presenting with an AMI who receive interventional or thrombolytic reperfusion must do so within 12 h of the onset of chest pain (170,171). Given the crucial events that occur in the first few minutes of reperfusion (oxidative stress, calcium overload, and mitochondrial permeability transition pore opening), any cardioprotective strategy must be applied prior to opening the infarct-related coronary artery. Accordingly, RIC given to patients in the ambulance while in transit to the interventional center have demonstrated a beneficial effect (28).

With late presentation, the infarct will have been completed, and the patient will derive little benefit from either intervention or an adjunct to reperfusion. Early presentation and revascularization will lead to small myocardial infarcts, and this patient will have little advantage from adjunctive therapy. There is a “sweet spot,” probably between 3 and 8 h from time of symptom onset to time of reperfusion, for adjunctive therapies to demonstrate maximal benefit.

COMORBIDITIES AND COMEDICATIONS. In preclinical studies, age (172) and comorbid diseases (126), such as hyperlipidemia, diabetes, and hypertension, which require a more robust conditioning signal, raise the threshold for protection. This raised cardioprotective threshold reflects fundamental molecular alterations within the heart, affecting both sensitivity to ischemia/reperfusion injury and response to a particular cardioprotective strategy (126,172-174). Unfortunately, most experimental models use healthy young animals, free of any comorbidities (175). Experimental studies using human atrial muscle from patients undergoing CABG, from aged and diabetic patients and patients with heart failure (176-178) confirmed the effect of comorbidity on the conditioning threshold and demonstrated resistance to various conditioning strategies.

Pharmacological therapy also impacts cardioprotection. Specific sulfonylureas used to treat type 2 diabetes can attenuate the conditioning response (134). Conversely, insulin, metformin, some statins, angiotensin-converting enzyme inhibitors, antiplatelet agents, and opioids can themselves be cardioprotective and raise the threshold for an additional benefit (64,173,179-181). A number of pharmacological agents used during cardiopulmonary bypass surgery interfere with the cardioprotective efficacy of RIC. Volatile anesthetics, such as isoflurane, and the intravenous anesthetic, propofol, either themselves confer cardioprotection or interfere with RIC through down-regulation of cardioprotective signaling (46,48). Intravenous nitroglycerine, nitroprusside, and opioid analgesics, each protective in experimental settings, also interfere with the apparent cardioprotective efficacy of a study intervention (173,180).

Taking these confounders into consideration in the design of any clinical study investigating RIC is hugely important; we must either design a study that does not use these agents (which may be impractical) or ensure that it is adequately powered and properly randomized.

EFFECTS OF RIC ON THE BLOOD AND VASCULATURE

Platelet activation is both a consequence and a driver of ischemia/reperfusion injury. Local ischemic preconditioning attenuates platelet activation and aggregation (182). In humans, marked systemic platelet
activation has been demonstrated in patients with acute coronary syndromes (183) or acute limb ischemia (184). In animal models, the extent of platelet activation is related to the extent of subsequent tissue injury after reperfusion (185). Indeed, blockade of platelet aggregation alone can significantly attenuate reperfusion injury. In healthy male volunteers subjected to 20-min forearm ischemia (160), platelet activation (measured by increased circulating monocyte-platelet aggregates) persisted up to 45 min, but was completely abolished in subjects randomized to receive RIPC prior to the ischemic insult. In patients with known obstructive coronary artery disease (186), RIPC prior to exercise stress testing reduced ADP-stimulated platelet aggregation. Similarly attenuated platelet aggregation was seen in patients undergoing ablation for atrial fibrillation when receiving RIPC (187). However, the potential clinical benefit of any of these findings remains to be seen.

Circulating monocytes play a key role in ischemia/reperfusion injury. RIC down-regulated the expression of a broad portfolio of proinflammatory genes in circulating monocytes (161). The functional importance of these gene expression changes was demonstrated by reduced neutrophil adhesion over 10 days of daily RIC (188). Neutrophil phagocytosis was not significantly altered at 24 h, but was suppressed after 10 days of RIC. In patients undergoing CABG (189), RIC was not associated with any difference in circulating markers of inflammation (e.g., interleukins 6, 8, or 10, or tumor necrosis factor-α levels) but neutrophil kinase beta-1 and beta-2 receptor expression was significantly reduced, confirming similar results in healthy human volunteers subjected to RIC (190).

The RIC stimulus is associated with coronary vasodilation in animal models (191) and peripheral vasodilation in the contralateral limb of human subjects undergoing RIC (192). In Kharbanda’s original description (17), RIPC by 3 cycles of 5-min ischemia/5-min reperfusion in the forearm provided potent protection against the endothelial dysfunction induced by 20-min ischemia/reperfusion in the contralateral arm. Using the same model, RIPC was not only effective immediately, but also induced a second window of protection against endothelial dysfunction at 24 h (44). When the RIC protocol was performed on the contralateral arm during the ischemia phase, but prior to reperfusion, both RIPC and remote ischemic per-conditioning were blocked by pre-treatment with the ATP-dependent potassium channel blocker, glibenclamide (193). Compared with young volunteers, elderly hypertensive subjects benefitted more from RIC, whereas basal levels of flow-mediated dilation were significantly greater in the younger population (194). RIC in healthy young subjects, repeated daily for 7 days (195), was associated with progressively improved flow-mediated dilation and cutaneous vascular conductance (a measure of microcirculatory function), which was sustained at 8 days after the cessation of RIC. In a subsequent study (196), similar beneficial effects persisted after 8 weeks of repeated RIC treatments. This prolonged effect of RIC on endothelial function was also observed in patients with AMI undergoing PCI (197). Endothelial function was tested at baseline, within 3 h, and then on days 2 and 7 post-procedure in 48 patients randomized to PCI with or without RIPC. Endothelial function improved early after treatment and was sustained 7 days after the intervention. Whether this was a primary effect of sustained modification of endothelial function or a secondary phenomenon, resulting from less systemic inflammatory reaction, is unknown. Likewise, 1 week of twice-daily limb RIC improved ATP-recruitable coronary blood flow velocity reserve in a small cohort of healthy volunteers and patients with heart failure (198).

**CONCLUSIONS AND PERSPECTIVE**

Solid evidence from experimental and clinical studies supports protection by RIC from ischemia/reperfusion injury of the heart and other organs (16). Details of the mechanisms for local release of the protective signal at the remote site and the contributions of neuronal and humoral pathways are not yet clear, not only in signal release, but also in signal transfer to the target organ and protective signal transduction within the target organ. Repeated brief inflation/deflation of a blood pressure cuff at the arm, leg, or both is easily feasible, noninvasive, inexpensive, effective, and safe. Ongoing trials will reveal whether the benefit in clinical outcome reported from small proof-of-concept trials where clinical outcome was not the primary endpoint (199) will really hold true (200,201).

Thus far, translation of cardioprotective strategies from successful experiments to the clinic has been somewhat disappointing, for reasons that have been highlighted elsewhere (64,166,167,202): premature enthusiasm for experimental data that were not unequivocal and not confirmed in larger mammalian models; poor clinical trial design; and lack of consideration for patients’ multiple comorbidities and comedications (126). The pharmaceutical industry has, understandably, largely given up on development of cardioprotective agents, because they may...
need to be given only once in the situation of acute ischemia/reperfusion, but not as continuous therapy.

It appears reasonable to focus on mechanical protection of the heart and other organs by RIC and to optimize protocols. Apart from RIC algorithm optimization (number/duration of ischemia/reperfusion cycles), a better mechanistic understanding of the underlying signal transduction will be necessary to overcome the confounding impact of comorbidities and comediations. RIC may then, indeed, be the future of cardioprotection (203).

Future investigations should explore the potential benefit of RIC, not only in patients with large evolving myocardial infarctions, but also in patients with cardiogenic shock and severe arrhythmias, including cardiac arrest and threatening global ischemia of the brain, heart, liver and kidney during organ transplantation and extensive cardiovascular surgery.

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KEY WORDS acute myocardial infarction, coronary artery bypass grafting, myocardial ischemia, reperfusion
**Aβ Amyloid Pathology Affects the Hearts of Patients With Alzheimer’s Disease**

**Mind the Heart**

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**ABSTRACT**

**BACKGROUND** Individually, heart failure (HF) and Alzheimer’s disease (AD) are severe threats to population health, and their potential coexistence is an alarming prospect. In addition to sharing analogous epidemiological and genetic profiles, biochemical characteristics, and common triggers, the authors recently recognized common molecular and pathological features between the 2 conditions. Whereas cognitive impairment has been linked to HF through perfusion defects, angiopathy, and inflammation, whether patients with AD present with myocardial dysfunction, and if the 2 conditions bear a common pathogenesis as neglected siblings are unknown.

**OBJECTIVES** Here, the authors investigated whether amyloid beta (Aβ) protein aggregates are present in the hearts of patients with a primary diagnosis of AD, affecting myocardial function.

**METHODS** The authors examined myocardial function in a retrospective cross-sectional study from a cohort of AD patients and age-matched controls. Imaging and proteomics approaches were used to identify and quantify Aβ deposits in AD heart and brain specimens compared with controls. Cell shortening and calcium transients were measured on isolated adult cardiomyocytes.

**RESULTS** Echocardiographic measurements of myocardial function suggest that patients with AD present with an anticipated diastolic dysfunction. As in the brain, Aβ40 and Aβ42 are present in the heart, and their expression is increased in AD.

**CONCLUSIONS** Here, the authors provide the first report of the presence of compromised myocardial function and intramyocardial deposits of Aβ in AD patients. The findings depict a novel biological framework in which AD may be viewed either as a systemic disease or as a metastatic disorder leading to heart, and possibly multiorgan failure. AD and HF are both debilitating and life-threatening conditions, affecting enormous patient populations. Our findings underline a previously dismissed problem of a magnitude that will require new diagnostic approaches and treatments for brain and heart disease, and their combination. (J Am Coll Cardiol 2016;68:2395–407) © 2016 by the American College of Cardiology Foundation.
Heart failure (HF) and Alzheimer’s disease (AD) are age-dependent diseases that are growing worldwide. HF claims 36% of cardiovascular deaths, with an aging prevalence growth of 4% to 9% from 60 to 80 years of age, and AD is the fifth most common cause of death in patients 65 years of age and older (1). Epidemiological evidence indicates that HF shares risk factors with dementia through analogous genetic and biochemical profiles, and common triggers (2–8). Additionally, a number of more recent discoveries suggest a closer common pathogenesis between the 2 conditions. These include the discovery that protein aggregates deposit in the myocardium of patients affected by idiopathic dilated cardiomyopathy (iDCM) (9), and that such deposits are biochemically akin to those found in AD (10). Moreover, genetic variants in the same gene associated with early-onset AD (presenilin = PSEN) were reported in familial (11) and sporadic cases (9) of iDCM. Thus, whether these conditions are causally linked or part of a multiorgan syndrome, their potential coexistence raises an alarming prospect with people living longer.

Even though “cardiogenic dementia” was first postulated nearly 4 decades ago (12), and numerous studies have identified HF as a risk factor for AD (13,14), it is unknown whether AD affects myocardial function and if the 2 maladies share a common pathogenesis. The prevailing belief is that major determinants of the heart-to-head connection are compromised blood flow to the brain, amyloid, or atherosclerotic angiopathy (15–17). The cognitive decline from low brain perfusion has been shown early in pre-symptomatic AD, whereas increasing blood flow to the brain improves AD symptoms (15). Whether the opposite is true, namely compromised heart function in patients affected by AD, in the absence of other underlying cardiovascular disease, is unknown.

A pathological hallmark of AD is the accumulation of amyloid deposits in the form of extracellular plaques (18) (composed of the amyloid precursor protein [APP] proteolytic fragments [Aβ]) (19,20) in the brain parenchyma, causing neuronal cell death (21). Abnormal cleavage of APP (22) leads to an amyloidogenic pathway, generating pathological Aβ fragments.

Here, we investigated whether Aβ amyloid accumulates in the hearts of AD patients, affecting organ function. We found that: in vivo myocardial and in vitro cardiomyocyte function are compromised in AD patients; Aβ140 and Aβ132 are both present in the myocardium, and are increased in the hearts of AD patients. These findings, in combination with our previous report of the toxic effect of Aβ pre-amyloid oligomers (PAOs) on cardiomyocytes (9), suggest Aβ amyloid as a novel pathogenesis for myocardial dysfunction.

METHODS

Detailed methods are available in the Online Appendix.

HUMAN SUBJECTS. A cohort of AD cases and controls was selected from the Beth Israel Deaconess Medical Center clinical database to determine in vivo myocardial function in AD. Fresh heart and brain specimens from a separate cohort of patients with clinical diagnosis of AD and controls were used for in vitro pathological and functional studies.

TISSUE SAMPLES. Myocardial tissue samples were fixed in 4% paraformaldehyde or 2% glutaraldehyde for imaging. Frozen myocardial and brain samples were used for imaging and molecular tests. Fresh tissue was used to isolate adult left ventricular (LV) cardiomyocytes (23,24).

IMAGING. Brain sections were stained for amyloid fibers with Thioflavin S or silver stain, and for PAO by immunohistochemistry using structural antibodies (A11). Transmission electron microscopy (TEM) was used to visualize the fibers in the myocardium. Immunogold TEM with structural antibodies against Aβ12-PAO (VIA antibodies, recognizing the last 3 amino acids Val40-Ile41-Ala42 of Aβ(12) (25) were used to identify Aβ in the myocardial deposits.

MOLECULAR TESTS. Immunoblotting and enzyme-linked-immunosorbent assays (ELISA) were used to identify and quantify Aβ peptides in heart and brain tissue.

CARDIOMYOCYTE FUNCTION. Adult LV cardiomyocytes were isolated by enzymatic digestion (24,26). Contractility and calcium (Ca2+) transients were measured using a video edge-detection and dual-excitation system (Ion-Optix, Westwood, Massachusetts) (27).

RESULTS

DIASTOLIC FUNCTION IS REDUCED IN AD. Reduced blood flow to the brain from low cardiac output has been linked to cognitive impairment in HF. Here, we analyzed whether, conversely, myocardial function is
between AD status and cardiac function for age. We found that age predicts a reduction in diastolic function by mitral valve E/A ratio (MVE/A) (Online Table 3A), as previously shown (28). We then performed exploratory analyses of means and standard errors of the mean by age category and AD status. Cases and controls were divided into 3 age groups (<65, 65 to 80, and >80 years of age). The Student t test analysis, as well as the estimates obtained by ordinary least squares of our linear regression model (Online Table 3B), showed no significant differences between AD and MVE/A ratio. Notably, diastolic function did not differ in the third tertile of age in AD versus controls (MVE/A ratio 0.82 ± 0.08 vs. 0.86 ± 0.08). However, a correlation between AD and anticipated decline of myocardial function was present in the first age tertile (1.20 ± 0.18 vs. 1.34 ± 0.12) (Figure 1A). This appeared to be better explained by deficits in myocardial compliance because such a pattern was not shown for the atrial component (A) of the MVE/A (Figure 1B). Furthermore, we also used a linear regression F test (p < 0.05), which showed that the pattern we observed was consistent with poorer diastolic function at earlier ages among the AD participants, conforming with the pathogenesis of AD as a disease of anticipated aging.

AD status was also associated with increased LV wall thickness, as shown in other infiltrative diseases, including classical cardiac amyloidosis. However, the difference was significant only in elderly subjects (left ventricle septal wall thickness 1.12 ± 0.05 vs. 1.01 ± 0.04; p < 0.05 left ventricle inferolateral wall thickness 1.07 ± 0.05 vs. 1.00 ± 0.03; p < 0.05), but not in the first age tertile, and therefore, not a possible cause of the anticipated diastolic defect found in AD subjects younger than 65 years of age (Figures 1C and 1D). Similarly, aortic valve peak velocity was increased only in older AD subjects (2.44 ± 0.41 vs. 1.51 ± 0.14; p < 0.05), suggesting it as a putative cause of wall thickening at later time points, but, again, unlikely to be a determinant of early diastolic impairment. The analysis of individual cases showed that 4 AD patients had high values of peak aortic velocity, indicating a stochastic occurrence of associated reduced aortic valve compliance in a few older cases.

Clinical assessment of myocardial function (Online Tables 2A to 2C) did not show any significant difference between AD cases and controls. Because AD is an age-related disease, we then performed a linear regression analysis and adjusted the associations

### Table 1: Clinical Characteristics of AD Cohort Cases

<table>
<thead>
<tr>
<th>Control (n = 35)</th>
<th>AD (n = 22)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age, yrs</td>
<td>78 (67-83.8)</td>
</tr>
<tr>
<td>Male</td>
<td>17 (48.6)</td>
</tr>
<tr>
<td>Caucasian ethnicity</td>
<td>35 (100.0)</td>
</tr>
<tr>
<td>Medical history</td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td>2 (6.3)</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>5 (15.6)</td>
</tr>
<tr>
<td>Neoplasia</td>
<td>10 (31.3)</td>
</tr>
<tr>
<td>High blood pressure readings</td>
<td>4 (12.5)</td>
</tr>
<tr>
<td>Atrial fibrillation</td>
<td>7 (21.9)</td>
</tr>
<tr>
<td>Cerebrovascular accidents</td>
<td>1 (3.1)</td>
</tr>
<tr>
<td>Medications</td>
<td></td>
</tr>
<tr>
<td>Aspirin</td>
<td>12 (37.5)</td>
</tr>
<tr>
<td>NSAIDs</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Oral anticoagulants</td>
<td>9 (28.1)</td>
</tr>
<tr>
<td>Antiangregant agents</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Statins</td>
<td>8 (25.0)</td>
</tr>
<tr>
<td>Antiarrhythmical agents</td>
<td>5 (15.6)</td>
</tr>
<tr>
<td>Digoxin</td>
<td>4 (12.5)</td>
</tr>
<tr>
<td>β-Blockers</td>
<td>13 (40.6)</td>
</tr>
<tr>
<td>Calcium-channel blockers</td>
<td>2 (6.3)</td>
</tr>
<tr>
<td>ACE inhibitors</td>
<td>2 (6.3)</td>
</tr>
<tr>
<td>ARBs</td>
<td>1 (3.1)</td>
</tr>
<tr>
<td>Diuretic agents</td>
<td>6 (18.8)</td>
</tr>
<tr>
<td>Levothyroxine</td>
<td>7 (21.9)</td>
</tr>
<tr>
<td>Any ACh inhibitors</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Donepezil</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Rivastigmine</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Galantamine</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Memantine</td>
<td>0 (0)</td>
</tr>
<tr>
<td>SSRI/SNRI</td>
<td>3 (9.4)</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>1 (3.1)</td>
</tr>
</tbody>
</table>

Values are median (interquartile range) or n (%). Demographic features, clinical presentation, and drug prescriptions recorded at the time of cardiac ultrasound. ACE = angiotensin-converting enzyme; AChE = acetylcholinesterase; AD = Alzheimer’s disease; ARB = angiotensin-receptor blocker; NSAID = nonsteroidal anti-inflammatory drug; SNRI = selective norepinephrine reuptake inhibitor; SSRI = selective serotonin reuptake inhibitor.

abnormal in AD. We studied a cohort of AD patients with the clinical diagnosis or diagnostic workup of AD, in the absence of other underlying conditions affecting myocardial function (including history of coronary artery disease [CAD], previous myocardial infarction, hypertension, primary or secondary amyloidosis, dilated/hypertrophic cardiomyopathy, endocarditis, chemotherapy, or radiotherapy). Cases and controls were matched by age/sex/ethnicity with a 1:2 ratio (Table 1, Online Table 1, Online Figure 1).

Clinical assessment of myocardial function (Online Tables 2A to 2C) did not show any significant difference between AD cases and controls. Because AD is an age-related disease, we then performed a linear regression analysis and adjusted the associations

CLINICAL ASSESSMENT OF THE PATIENT POPULATION UNDERLINED THE ROLE OF BETA-BLOCKADE IN PROTECTION FROM COGNITIVE DYSFUNCTION. Associated diseases were similarly distributed, with an equivalent number of cases with the diagnosis of diabetes, arrhythmia (particularly atrial fibrillation, which often occurs in the aging population), and neoplasms (Online Table 4). Interestingly, only 1 AD
case was on beta-blocker therapy. Instead, about one-third of controls were on such treatment. Beta-blocker therapy has been associated with reduced risk of AD (29). Although beta-blockade may improve diastolic function, skewing our results, it does so when it achieves a significant reduction in heart rate, which was unchanged in our control versus AD cases. However, we performed analysis of variance to account for this variable, and separated the controls into 2 groups on the basis of the presence or absence of beta-blocker therapy. We found that there was no difference in diastolic function, as measured by MVE/A, between the 2 therapeutic regimen groups (Online Tables 5A and 5B).

AD TISSUE SAMPLES. The definitive diagnosis of AD versus other forms of dementia can only be made at pathological and histological examination. Therefore, we collected samples of heart and, when possible, brain tissue from 4 cases with a medical history of AD from which the patients were maintained in respiratory support (Table 2). This stringent criterion is required to avoid postmortem false-positive depositions of protein aggregates, but made the procurement of such samples extremely rare. We were, therefore, limited to the following few, but highly selected and significant, cases: 1) a 58-year-old Caucasian woman with a history of cognitive impairment for 10 years and diagnosis of AD for 7 years; 2) a 70-year-old Caucasian woman with a clinical diagnosis of dementia. In this case, the clinical diagnosis of AD was confirmed at pathology by CERAD (Consortium to Establish a Registry for AD) plaque score C and Braak tangle score IV (30,31); 3) an 84-year-old Caucasian

**FIGURE 1** Crude Means of Cardiac Ultrasound Parameters for Groups and Age Ranges Show Worse Myocardial Function in the AD Clinical Cohort Compared With Controls

Crude means of cardiac ultrasound parameters for age tertiles and groups corresponding to the linear regression. The graph in A shows how the value of the MVE/A ratio is lower in younger AD subjects and progressively overlaps with the controls at advanced ages. The mean value of the MVE/A ratio of AD patients in the first tertile appears intermediate between the value of the first and the second tertile of controls. An analogous pattern is shown for AD patients in the second tertile (A). (B) Notably, the atrial (A) component alone showed no differences in all groups, suggesting that the LV compliance is the main contributor to the diastolic dysfunction in AD patients. (C and D) show the increased LV septal and inferior wall thickness, respectively, in the older AD subjects. *p < 0.05. Blue diamonds indicate controls; orange squares indicate AD cases. Aβ = amyloid beta; AD = Alzheimer’s disease; LV = left ventricle/ventricular; MVE/A = mitral valve E/A ratio; yo = years of age.

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JACC Vol. 68, No. 22, 2016
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woman with a clinical diagnosis of AD for 3 years. The heart from this case was removed 7 h and 30 min postmortem (labeled as tissue donor); and 4) a 91-year-old Caucasian man with a clinical diagnosis of AD.

**STRUCTURAL STAINING IDENTIFIED Aβ IN THE MYOCARDIUM.** Neuritic plaques are distinctive histological lesions required for the diagnosis of AD. We therefore imaged brain and heart tissue from the 4 pathological specimens of AD compared with controls.

First, we verified the presence of Aβ for the diagnosis of AD in the brain specimens by immunostaining with structural A11 antibodies (32,33) and staining with Thioflavin S (34), which revealed the presence of pre-amyloid and amyloid deposits, respectively (Figures 2A and 2B). The brain of the 70-year-old woman showed plaques by Hirano silver staining (Figures 2E and 2F), and the typical global atrophy at gross pathology (Figure 2G).

In the heart, small intracellular or extracellular amyloid deposits cannot be clearly visualized by traditional staining due to the high background generated by the birefringent sarcomeres. Therefore, we imaged the hearts by TEM and identified fibrillar deposits in AD myocardial specimens (Figure 3, Online Figure 2). However, the fibrillar-rich content of interstitial tissue in the myocardium also hinders the recognition of pre-amyloid and amyloid deposits. Therefore, to identify the pathological inclusions within the normal extracellular fibrillar matrix, we used immunogold TEM on the specimens from the oldest and the youngest cases. We used anti-Aβ42 antibodies and/or Aβ42-PAO structural VIA antibodies. Positive VIA immunogold staining was detected within the muscle fibers and the interstitial tissue (Figure 4). These data indicate that inclusions of pathological Aβ are present in the myocardium of AD patients. By contrast, the signal for non-oligomerized Aβ42 was below the threshold of the signal noise; therefore, we cannot infer an evaluation of the presence of the nonoligomerized form of the peptide using this method.

**MOLECULAR TESTS IDENTIFIED BOTH Aβ40 AND Aβ42 IN THE MYOCARDIUM.** The presence of both Aβ peptides (Aβ40 and Aβ42) was confirmed and quantified by immunoblotting and ELISA. Given the difference of only 2 amino acids between Aβ40 and Aβ42, we first determined the antibody specificity by dot blot. We confirmed that each antibody uniquely recognizes the corresponding peptide (Online Figure 3). By immunoblotting, we then found that both Aβ peptides (molecular weight 5 to 10 kDa) are present in the heart and brain of AD patients (Figure 5A, Online Figure 4).

ELISA confirmed that Aβ40 and Aβ42 are expressed in the heart, although at lower levels than the brain. In both organs, Aβ40 and Aβ42 levels were increased in the tissue from AD patients compared with controls (Figure 5B). These data further indicate that, similar to the brain, Aβ is present in the myocardium and is increased in AD.

**CARDIOMYOCTES DISPLAY RELAXATION DEFECTS.** To test whether the relaxation defect identified clinically is solely due to interstitial deposits and extracellular matrix stiffness, or if cardiomyocyte function is also abnormal in AD, we measured contractility and Ca⁺⁺ transients in LV cardiomyocytes isolated from the 58-year-old AD heart, compared with cardiomyocytes isolated from 68- and 56-year-old male patients (Table 1).
FIGURE 2  Aβ Is Present in AD Brain Specimens at Histopathology

Immunohistochemistry images of brain plaques stained with Thioflavin S and structural anti-oligo Aβ1 antibodies in the brain of the (A) 91-year-old man and (B) the 84-year-old woman. The images show minimal overlap of the Aβ anti-oligo antibodies with the fibrillar mature plaques, as described by Kayed and Glabe (33). (C and D) Control brains. (E, F, G) Case of a 70-year-old woman. (E) Silver staining of the occipital cortex, showing amyloid plaques and amyloid angiopathy by Hirano silver staining. (F) Hematoxylin-eosin staining for the brain structure. Magnification 200×. (G) Gross pathology of the brain. The middle image shows the entire brain, with the hemorrhagic frontal lobe of the right hemisphere. The left and right images show the brain sections of the posterior and anterior halves of the brain, respectively. The left hemisphere appears hypotrophic, a common feature of AD. Magnification 100×. CF = Caucasian female; CM = Caucasian male; H/E = hematoxylin and eosin; PAO = pre-amyloid oligomer; other abbreviations as in Figure 1.
control hearts (Online Figure 5). AD cardiomyocytes displayed slower velocities of relaxation (time to 50% relaxation 0.272 vs. 0.238) and prolonged Ca^{2+} transients (time to peak 0.544 vs. 0.151; time to 90% relaxation 0.328 vs. 0.084) compared with controls, indicating a defect in Ca^{2+} homeostasis. No statistical calculations were performed because we only measured 3 cardiomyocytes from 1 AD case. Although we cannot infer a role for circulating Ab versus a primary defect in AD cardiomyocytes, it is possible that cardiomyocytes and extracellular infiltrates may combine to determine the overall functional defect in AD hearts.

DISCUSSION

Dilated cardiomyopathy (DCM) and HF have been recently added to the growing list of age-related proteinopathies, of which AD is one of the founders. DCM shares several risk factors with dementing disorders, including AD, and clinical evidence links DCM and AD through analogous epidemiological, biochemical, and genetic profiles. Here, we provide novel evidence that the heart and brain can be affected within the same clinical picture. We found that diastolic dysfunction is an early defect in patients affected by AD, and that intramyocardial deposits of Aβ are present in the myocardium of these patients. Because Aβ-PAs affect cell function in the heart as in the brain, it is possible that they may contribute to the observed myocardial dysfunction.

HEAD-TO-HEART CONNECTION IN AD: A MULTIFACETED MECHANISM. HF has been found to be a risk factor for both vascular dementia and AD, and cognitive impairment has been found in patients with HF and correlates with its severity. This relationship has been attributed to reduced blood flow to the brain and diffuse amyloid angiopathy. The latter is also regarded as the reverse link between myocardial functional decline in AD and aging. Furthermore circulating Ab was shown to worsen vascular atherosclerosis, and to predict disease progression and cardiovascular mortality in patients with established CAD. Here, we tested whether other mechanisms link cardiac dysfunction to the brain disorder in the absence of any known causes of compromised cardiovascular function. This possibility was suggested by our recent discoveries of: 1) protein aggregates in iDCM; 2) common genetic mutations in AD and iDCM; and 3) common components of the AD and iDCM aggregates. Thus, low perfusion and amyloid angiopathy may not be the sole contributors to the heart-to-brain connection, and the possibility that patients with AD would present with myocardial dysfunction was a reasonable hypothesis. Although the small number of cases for a clinical assessment limits the statistical significance of our findings, our data suggest that AD patients may also present with compromised diastolic cardiac function, raising the awareness of a growing clinical problem.

ANTICIPATED DIASTOLIC DYSFUNCTION OCCURS IN PATIENTS WITH CLINICAL DIAGNOSIS OF AD. Reduced diastolic function is a normal event in myocardial aging. Although AD has a close correlation with age, and diastolic function in AD progressively...
worsen with age, we found that AD patients displayed worse values at younger ages compared with controls, asymptotically approaching the values of old-age controls. This finding supports the definition of the AD phenotype as anticipated aging. Larger datasets are, however, necessary to unequivocally establish a direct link between AD and myocardial dysfunction. Although the pathogenesis of diastolic dysfunction is complex, diastolic dysfunction is a common feature associated with myocardial hypertrophy. In our AD cohort, a significant thickening of the left ventricular walls was observed only in the oldest individuals, and therefore was unlikely to be the cause of the observed diastolic dysfunction in the younger cases. This would most likely be due to the occurrence of aortic stenosis in elderly AD cases. Thus, neither hypertrophy nor aortic valve stenosis can be the underlying cause of the diastolic dysfunction observed in the younger AD cohort. Other mechanisms linked to the pathogenesis of AD may account for the observed changes, including the accumulation of Aβ in the myocardium.

**Aβ INCLUSIONS ARE PRESENT IN MYOCARDIAL TISSUE IN AD.** Deterioration of cognitive functions characterizes a number of dementing processes, including “senile dementia.” AD instead defines a distinct condition of “pre-senile” dementia for which the definitive diagnosis can only be made at pathology. In our clinical cohort, in 1 case who was deceased, the diagnosis was also confirmed by gross pathology and histological analysis. Three cases
presented with positive family history for AD. Although these data strengthen the confidence in the diagnosis of our cohort of AD patients, we further tested whether, in fact, AD pathology extends outside the brain by determining the presence of Aβ in cardiac muscle tissue samples harvested from a separate cohort of deceased AD patients.

Aβ fragments generated from abnormal cleavage of APP (Online Figure 6) (22) are among the main constituents of senile plaque and cerebrovascular deposits in AD. Aβ aggregates typically deposit, not only in the brain, but also in the submeningeal vasculature in patients affected by AD, causing damage to the vascular wall (44). Such deposits have been attributed to either basolateral-to-apical transport of soluble Aβ or disruption of the blood-brain barrier and Aβ leakage into the bloodstream, as shown in human and mouse models of AD (45,46). Flowing in the bloodstream, Aβ may deposit in distal organs and vessels, causing other forms of non-neurological amyloid pathology, as has been reported in the skeletal muscle, skin, kidney, lung, and intestine of AD patients (47), but also at distance, in the heart and brain, causing AD

(A) Immunoblotting of AD and young and age/sex/ethnicity-matched control hearts and brains. Samples are ordered by age, from younger to older. The blot demonstrated the presence of both Aβ40 (top) and Aβ42 (bottom) in the heart and brain of AD patients. Aβ fragments of multiple molecular weights (MW) (≥30, 40, 50 kDa) could be detected both in the heart and the brain. Of note, both Aβ40 and Aβ42 high MW bands were also present in old cases without diagnosis of AD, and also in young patients, although at lower levels, as previously described in the brain. (B) Quantification of the levels of Aβ40 and Aβ42 in AD and donor samples by enzyme linked immunosorbent assay, showing increased levels of both fragments in the heart and brain of patients affected by AD. Controls were divided by age ≥ or ≤50 yrs. Student t test *p < 0.05, **p < 0.01, and ***p < 0.005 compared with AD. A 1-way analysis of variance (ANOVA) was also performed in all groups, followed by Bonferroni post hoc analysis. ANOVA was significant for Aβ40 (p < 0.0001) and Aβ42 (p < 0.05). Post-test for Aβ40 was significant (p < 0.001) by age group comparisons, whereas p = 0.074 for all groups for Aβ42. The Levene test was performed for equal variances. A larger sample size may be necessary to establish the significance of the differences. The axis of ordinates is separated to better visualize the control values. Values indicate pg/mg Aβ/total protein. Abbreviations as in Figure 1.
and DCM (49,50). Thus, the deposition of amyloid fibers in the heart from circulating Aβ peptides, leading to cardiac dysfunction in AD patients, is a likely event. Notably, a significant amount of Aβ was identified in the peripheral circulation and platelets, worsening CAD. Thus, it is possible that accumulation of Aβ in the heart compromises myocardial function in patients with AD, identifying a more profound pathogenic root for the brain-to-heart connection, in addition to the effect of Aβ on vascular atherosclerosis or to other mechanisms linked to amyloidosis, including neurohormonal activation and inflammation. This possibility is supported by our previous data indicating a similar mechanism of toxicity of PAOs on cardiomyocytes (9), as demonstrated in neurons (37). Additionally, amyloid proteins, including Aβ, have been shown to directly activate specific proinflammatory and necrotic responses (inflammasome and necrosome). Thus, Aβ may also affect myocardial function through the activation of the newly discovered functional amyloid signaling (51).

**MYOCARDIAL DYSFUNCTION IN AD: A POSSIBLE METASTATIC OR SYSTEMIC PROCESS.** Various pathogenic mechanisms can be invoked to explain our findings: either Aβ fragments may deposit in the heart as in other distal tissues, recreating the amyloid pathology as a metastatic disease; or a common
genetic, biochemical, and molecular profile and common triggers may affect both organs as a systemic disease; or both (Central Illustration). Although the genotype was not available to exclude the presence of known AD mutations, our cohort of AD cases was predominantly sporadic, suggesting the absence of Mendelian genetic mutations, but not excluding predisposing genetic variants or polymorphisms.

**STUDY LIMITATIONS.** We acknowledge the following limitations of this study.

1. The number of samples available for the clinical, pathological, and molecular biology assessment is limited. However, obtaining heart specimens from patients under respiratory support is extremely difficult, and we were fortunate to be able to obtain such specimens. Furthermore, case reports are important to raise the awareness of a potential clinical problem. The results from epidemiological cohorts generally have wide confidence intervals. Therefore, we cannot infer temporality on the basis of the cross-sectional nature of our validation cohort. However, the results of our analysis support the possibility of differences in cardiac outcomes by AD status. We also acknowledge the measurements of cardiomyocyte function were obtained only from 3 myocytes of the youngest case. Isolating adult human cardiomyocytes is challenging, and even more so from hearts with amyloid inclusions, limiting the technical success of the procedure.

2. The diagnosis of AD is anatomopathological. It is obtained from the following brain regions: the middle frontal gyrus (Brodman area [BA]8/9); superior temporal gyrus (BA22/21); inferior parietal lobule (BA39/40); occipital cortex, including 1 visual cortex (BA17/18); 1 motor/sensory cortex (BA4/1/2/3); and the hippocampus/entorhinal cortex. In our study, the brain samples were obtained from the cortex. However, given the mentioned difficulties in obtaining such highly selected heart and brain samples, and because, at the late symptomatic stage, Aβ pathology spreads outside the hippocampus and entorhinal cortex, the cortical regions we studied may be representative for the pathological diagnosis of AD. Furthermore, the diagnosis of 71-year-old case of AD followed the Aging-Alzheimer’s Association guidelines, and the heart of this case featured the same myocardial pathological changes as the other cases.

3. A detailed clinical assessment of cardiac function for the cases from which the specimens were obtained was not always available. Instrumental evaluation (e.g., echocardiography for myocardial function) is not performed in patients found unsuitable for organ explant, such as those with AD, due to age. Other causes of cardiac dysfunction were excluded in the cases we collected. Larger studies are, however, mandatory to assess the presence and type of cardiomyopathy in patients with AD, as well as to determine the impact and mechanisms.

4. We acknowledge that the E/E’ ratio is a more accurate index of diastolic function by Doppler echocardiography. However, the clinical data were obtained retrospectively, and this measurement is not part of the routine clinical diagnostic assessment. Future prospective research studies directed to assess diastolic function will be required. Those may include cardiac magnetic resonance imaging.

**CONCLUSIONS**

Overall, our data underscore the importance of recognizing the pathological involvement of the heart in AD, in addition to the known involvement of other organs (18,24–26). The clinical burden of AD has notably increased over the last decade, due to the aging of the population and the development of more effective therapies, prolonging lives. The increase in prevalence and improved survival of AD patients would lead to an expansion of the already high prevalence of cardiovascular disease, including CAD and now HF. As the heart is exposed to the free circulatory flow of aggregate-prone peptides, and no barrier segregates the organ, the involvement of this “nonimmunoprivileged” organ may carry a worse prognosis, as is the case with cardiac AL amyloidosis (52). If AD and HF alone are severe health threats, their combination will represent a clinical challenge requiring an interdisciplinary approach to patient management and treatment.

**ACKNOWLEDGMENTS** The authors thank Prof. Murray Mittleman at the Harvard School of Public Health and the Catalyst for assistance with the statistical analysis; Dr. Carmine Gentile for critical input to the manuscript; Roberto del Monte for help with the Central Illustration and for his unconditional support that made this work possible; and Maria Ericsson from the Harvard Electron Microscopy Core for help with electron microscopy.

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COMPETENCY IN MEDICAL KNOWLEDGE: The relationship between AD and HF is mediated, not only by abnormalities of tissue perfusion, neurohormonal activation, and inflammation, but also by factors that directly affect the functional properties of the myocardium.

TRANSLATIONAL OUTLOOK: Further studies are needed to define the dysfunctional cardiac phenotype observed in patients with AD, characterize proteomics and biomarkers common to the cardiomyopathies, and brain pathology that accompany aging, and explore the impact of therapeutic approaches to each disease on outcomes related to the other.

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KEY WORDS amyloidosis, cardiomyopathy, dementia, heart failure, protein aggregates

APPENDIX For expanded Methods and Results sections as well as supplemental tables and figures, please see the online version of this article.
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