Peer Review File

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Review Comments:

The authors raise the important point that at present, ante-mortem criteria are used in the analysis of post-mortem CMR, which may not be valid. While the study population is relatively small (36 controls and 19 with pathology), it is a good pilot project to address this deficiency. Therefore, I do recommend this manuscript for publication, with the suggested major revisions as follows:

Changes in manuscript highlighted yellow

RECOMMENDATIONS:

Comment 1. In the abstract, it states the total number of scans is 56; each subgroup has 36, 8, and 11 scans, which only totals 55.

Reply 1:

Thank you for your comment. There were 37 controls, 8 subacute myocardial infarctions and 11 pulmonary emboli

Changes in the text:

Section - Abstract

Lines 47 – 48

56 scans were selected: 37 (66.1%) controls, eight (14.3%) subacute myocardial infarctions and eleven (19.6%) pulmonary emboli.

Comment 2. The authors refer to this as a "proof-of-concept" study; rather, I would characterize it as a pilot study. The concept of measuring and defining cardiovascular pathology in post-mortem MRI has been proven as feasible in many previous studies. The novel aim of this study is to produce standardized cut-offs for these common diseases. The study population here is small, and larger numbers will be needed to confirm and clarify the guidelines; therefore, it makes more sense to refer to it as a pilot study.

Reply 2:

Thank you for your comment. We agree and have changed the study form proof-of-concept to pilot throughout the manuscript.

Changes in the text:

Lines 38, 60, 94, 260, 310, 337

Changed proof-of-concept to pilot

Comment 3. Line 129- the authors that the results of the autopsy were known at the time of CMR analysis. This is a potential confounding variable that needs to be addressed in the discussion.

Reply 3:

Thank you for your comment. We have now clearly clarified the issue of blinding in this study. CMR scans for this study were selected from our original blinded study (1), where we demonstrated the sensitivity and specificity of post-mortem CMR to identify causes of death as reported by conventional autopsy. For the current study, to ensure robustness of "novel" post-mortem CMR parameters/thresholds, the same investigators selected autopsy confirmed cases reported with either acute/subacute myocardial infarction, pulmonary emboli or no structural abnormality (control cases) and re-evaluated CMR scans. We agree this may be a confounding variable and as such, we have discussed it in the limitations.

1. Femia G, Langlois N, Raleigh J, Gray B, Othman F et al. Comparing conventional autopsy to post-mortem MR and CT in determining the cause of sudden and/or unexplained death. Forensic Science, Medicine and Pathology 2021 (In press).

Changes in the text:
Section - Methods
Line 103 – 109
CMR scans for this study were selected from our original blinded study (7), where we evaluated the accuracy of post-mortem CMR in identifying causes of death as reported by autopsy between October 2014 to November 2016. For the current study, the same investigators selected cases reported to have either acute or subacute myocardial infarction, pulmonary emboli or no structural cardiac abnormality (i.e., control cases) on autopsy. The investigators than re-evaluated the CMR scans for myocardial wall thickness, myocardial signal intensity and ventricular cavity area.

Section - Discussion
Lines 312 – 317
Cases were selected from our original blinded study that demonstrated the sensitivity and specificity of post-mortem CMR to identify causes of death (7); however, for this study, the investigators were aware of the results of the autopsy and CMR studies. Ultimately, this may be a potential confounding variable. That said, we felt that evaluating CMR parameters directly against the histopathologic "gold standard" i.e. autopsy would maximize accuracy of our results.

Comment 4. The authors interchange the terms "subacute myocardial infarction" and "fibrosis/scar". However, fibrosis/scar would histologically correspond with a remote myocardial infarction. In the methods (line 147) they define the histologic criteria that were used to define subacute MI, which included interstitial edema, coagulative necrosis, neutrophil infiltration, macrophages or lymphocytes, and/or collagen fibrosis. This definition is slightly unclear - if fibrosis is the predominant feature, then this would be a remote/chronic MI rather than subacute. Similarly, coagulative necrosis and neutrophil infiltration would correspond to an acute MI; granulation tissue with scant collagen, macrophages, and lymphocytes would correlate with subacute. The authors need to clarify the histologic definitions of This needs clarification; it may help for the authors to create a table listing the histologic findings in each case of "subacute" MI.

Reply 4:
Thank you for your comment. We agree that the term "fibrosis/scar" corresponds to a remote more chronic myocardial infarction. The term was written incorrectly as all eight patients were diagnosed with

either acute or subacute myocardial infarction by conventional autopsy; acute and subacute myocardial infarction have been defined by the presence of intracoronary thrombus and/or interstitial oedema, coagulative necrosis (pyknosis, karyorrhexis) or poly-mononuclear cell infiltration and macrophages, lymphocytes or scant collagen. Therefore, we have removed the term “fibrosis/scar” from the manuscript.

Changes in the text:
Removed fibrosis/scar from the manuscript and expanded the description of myocardial infarction with reference to Michaud et al.

Section - Methods
Lines 152 - 155
Acute and subacute myocardial infarction were defined by the presence of intracoronary thrombus and/or interstitial oedema, coagulative necrosis (pyknosis, karyorrhexis) or poly-mononuclear cell infiltration and macrophages, lymphocytes or scant collagen

Comment 5. Line 147 - identification of pathology was per standard autopsy criteria - not all autopsy criteria are clearly standardized, and cut-offs depend upon the reference source and the pathologist’s individual experience. The pathologic diagnostic criteria for cardiomegaly and right or left ventricle hypertrophy need to be clearly stated, to better clarify how individuals were classified in the control group. This is particularly important because, as per Table 1, 3 members of the control group had a medical history of diabetes mellitus, ischemic heart disease, and/or hypertension, which raises the concern that potential cardiac pathology was included in the controls. This could also be a potential confounding factor to be addressed in the discussion.

Reply 5:
Thank you for your comment. We now clarify that we report findings from a single high-volume center, where only one highly experienced Forensic Pathologist (NL) performed autopsies in all cases. Further, we have included the pathologic diagnostic criteria for cardiomegaly and left ventricle hypertrophy with reference to reported parameters. We do concede that the final diagnosis of ventricular hypertrophy was based on the experience of the pathologist and as such we have added this to the limitations.

Three patients in the control group had a history of diabetes, ischemic heart disease and/or hypertension but were reported to have died from non-cardiovascular causes of death (trauma, sepsis and toxins). In addition, histological examination did not reveal any evidence of structural heart disease or acute/subacute myocardial infarction and as such they were included in the control group. However, this could be a confounding factor and has also been discussed in the limitations.

Changes in the text:

Section - Methods
Lines 149 - 152
Conventional autopsy was performed in a single high-volume center by an experienced pathologist (NL) as described previously (7). Cardiomegaly was assessed with reference to published ranges

for post-mortem heart weight (8, 9). Left ventricular hypertrophy was judged by the pathologist at the post -mortem examination directed by published guidelines (10, 11)

Section – Discussion
Lines 310 – 312
The results of this pilot study are limited by a single center involvement and a relatively small number of scans. As such, we suggest that further validation of our proposed CMR parameters be performed in larger studies.

Section – Discussion
Lines 326 - 331
Three patients in the control group had a history of diabetes mellitus, ischemic heart disease and/or hypertension but the cause of death was non-cardiovascular (trauma, sepsis and toxins). Although there was non-specific fibrosis on histological examination, there was no evidence of structural heart disease or acute/subacute myocardial infarction. Never the less, this may be a confounding factor and needs to be considered.

Comment 6. Line 182- The post-mortem interval in this study was relatively short (ranging 2-4 days for all cases), An important limitation to comment on in the discussion would be the uncertainty about the validity of the proposed diagnostic guidelines as the PMI lengthens and post-mortem changes of decomposition and autolysis progress.

Replay 6: Thank you for your comment. We agree and have added this limitation to the discussion.

Changes in the text:

Section – Discussion
Lines 331 - 333
Finally, the time interval from death to post-mortem imaging/examination in our study was relatively short. However, there is uncertainty about the validity of our proposed diagnostic parameters with longer time interval and more advanced body decomposition.

Comment 7. Line 286 - I would not advise an image-guided percutaneous biopsy in the non-neoplastic setting, as there are potential issues related to mis-sampling that could be harder to detect. In the event of family objection to autopsy, it seems they would be unlikely to permit invasive biopsy techniques.
Reply 7: Thank you for your comment. We have removed the sentence from the discussion.

We removed the following sentences:
Detecting myocardial infarction by post-mortem CMR may still require a histological confirmation but in cases where autopsy cannot be performed, CMR may allow for less invasive measures such as image guided percutaneous biopsy.

Comment 8. Line 287- simple typos - “assessing” clinical severity; measuring ventricular area ratios “can” be a useful marker

Reply 8: Thank you for your comment. We have made the corrections

Changes in the text:

Section - Discussion
Line 304
We have added “assessing” and “can” to the following sentences
In support of this finding, an ante-mortem study found that an RV to LV area ratio on cardiac CT was accurate at identifying pulmonary emboli and assessing clinical severity (15). Therefore, measuring ventricular area ratios can be a useful marker to indicate RV and/or pulmonary artery pathology in patients with unexplained death who cannot undergo autopsy.

Comment 9. Line 312 - The final sentence is immaterial, as both conventional autopsy and post-mortem cardiac MRI are subject to inter- and intra-operator variability. Everything that relies on human interpretation is subject to variability; the autopsy benefits from direct gross and microscopic visualization of pathology, which makes it the gold standard.

Reply 9: Thank you for your comment. We have removed the sentence from the discussion.

Changes in the text:
We removed the following sentence:
Conventional autopsy was considered the gold standard for identifying the cause of death, but it is nevertheless subject to intra- and inter-operator variability.