Online data supplement

This data supplement has been provided by the authors to give the readers additional information about their work.

Supplement to: Vos A, van der Wal AC, Teeuw AH, Bras J, Vink A, Nikkels PGJ. Cardiovascular causes of sudden unexpected death in children and adolescents (0-17 years); a nationwide autopsy study in the Nederlands.

Table of contents

| Content                                              | Page |
|------------------------------------------------------|------|
| Supplemental methods: Cardiovascular autopsy         | 2    |
| Supplemental results: Cardiovascular findings of uncertain significance | 3    |
| Supplemental discussion: Borderline myocarditis      | 5    |
| Supplemental figure I: Borderline myocarditis        | 6    |
| Supplemental table I: Cardiovascular causes of death and structural normal hearts | 7    |
| Supplemental table II: Non-cardiovascular causes of death | 8    |
| References                                           | 9    |
Supplemental methods

Cardiovascular autopsy

Examination of the heart and great arteries included recording of heart weight, developmental abnormalities, measurement of the atrial and ventricular dimensions, myocardial thickness, structure and insertion of cardiac valves and aortic root including coronary ostia. Macroenzyme (LDH) staining of one entire biventricular myocardial slice was performed for detection of acute cardiomyocyte damage. Sections for histologic examinations were taken from both ventricles (in most cases a complete biventricular heart slice) and atrioventricular nodal area/interventricular septum and stained with haematoxylin and eosin (H&E).

If a myocarditis was suspected on routine H&E stained slides, additional CD3 and CD68 immunohistochemical stains were performed to detect T-lymphocytes and macrophages respectively. In cases of presence of focal infiltrates with only discrete myocardial damage, the case was categorized as borderline myocarditis.[1] Diffuse inflammatory infiltration with substantial myocardial damage was categorized as fulminant myocarditis.
Supplemental results

Cardiovascular findings of uncertain significance

In 13/56 autopsied cases (23%) a cardiovascular cause of death was found. Additionally, we found cardiovascular abnormalities of which the association with the death was uncertain.[2] These cardiovascular abnormalities of uncertain significance were present in 14 cases (25%). In one of these cases another cardiovascular disease was found to be the main cause of death. In 4 cases no other cause of death was found and the cases were classified as ‘no structural abnormalities’. In total, cardiovascular abnormalities (cardiovascular causes of death and cardiovascular findings of uncertain significance) were found in 26/56 cases. (46%)

Findings of uncertain significance included: small atrium septum defect (n=2), aberrant right subclavian artery/arteria lusoria (right subclavian artery originating from the descending aorta and running behind the esophagus to the right (n=2)), pulmonary venolobar (Scimitar) syndrome which was already diagnosed before death (n=1), abnormal outlet of the coronary sinus in the left atrium (n=1), a high take off of the left coronary artery with a collateral artery connecting the right coronary artery to the left without histological signs of acute ischemia (n=1), and borderline/focal myocarditis (n=8).

Borderline myocarditis

In 2/56 (4%) cases a fulminant myocarditis was found to be the cause of death. In one of these cases a parvovirus was detected in the myocardium. In another 8/56 cases (14%), age range 1 to 14 years, one or more clustered myocardial infiltrates of T-lymphocytes and macrophages, with focal indication of cardiomyocyte damage, were present (online supplemental figure I). These were diagnosed as borderline lymphocytic myocarditis according to the Dallas criteria.[1] Histologic examination of the remaining organs showed similar infiltrates in
larynx, trachea, lungs, tonsils, liver and/or brain in 7 of 8 cases. A cause of death, unrelated to
the focal myocarditis, was found in 5 of these cases (intussusception with pulmonary edema,
gangrenous purpura, brain herniation due to dysfunction of a ventricular-peritoneal shunt, and
pneumonia (2x)). The other three children died of unknown causes, since borderline
myocarditis is considered to be an uncertain cause of death.[2]
Supplemental discussion

Borderline myocarditis

We found limited focal lymphocytic myocarditis in 8/56 cases (14%), which, according to the recent guidelines of autopsy in sudden cardiac death, should be interpreted as at most an uncertain cause of death.[2] Our results, with an evident cause of death other than the borderline myocarditis in 5/8 cases, confirm this statement. Since we also found mild lymphohistiocytic infiltrates in the liver, lungs and/or brain in most of these cases (7 out of 8), this focal myocarditis most likely represents a generalized viral infection, as suggested by Bajanowski et al.[3] Unfortunately, systematic viral cultures or polymerase chain reactions of the myocardium to confirm this statement are lacking. Our results endorse the view to be cautious with interpreting a focal myocarditis as the cause of death. Especially since erroneous interpretation of insignificant findings at autopsy may masquerade the presence of an unnoticed inherited arrhythmogenic disease.[4]
Supplemental figure I: Borderline myocarditis

An example of a focus of lymphohistiocytic infiltrate with focal cardiomyocyte damage on H&E staining (A), with immunohistochemical analysis of the infiltrate. (CD3 positive T-lymphocytes in B and CD68 positive macrophages in C)
**Supplemental table I: Cardiovascular causes of death and structural normal hearts**

### Cardiovascular causes of death

| Age  | Sex | Cause of death                                                   | Circumstances of death                        |
|------|-----|-----------------------------------------------------------------|----------------------------------------------|
| 1 d  | ma  | Congenital aortic stenosis                                      | During sleep                                  |
| 2 d  | ma  | Complete atroventricular septal defect                          | During sleep                                  |
| 3 d  | fe  | Sudden Infant Death Syndrome                                    | During sleep                                  |
| 4 m  | fe  | Sudden Infant Death Syndrome                                    | During sleep                                  |
| 1.5 m| ma  | Acute decompensated heart failure                               | During bathing                                |
| 1.5 m| fe  | Fulminant myocarditis                                           | During feeding                                |
| 1.5 m| fe  | Sudden Infant Death Syndrome                                    | During sleep                                  |
| 2 m  | ma  | Sudden Infant Death Syndrome                                    | During sleep                                  |
| 2.5 m| fe  | Sudden Infant Death Syndrome                                    | During sleep                                  |
| 3 m  | ma  | Sudden Infant Death Syndrome                                    | During sleep                                  |
| 3 m  | fe  | Anomalous origin left coronary artery                            | During feeding                                |
| 3.5 m| ma  | Sudden Infant Death Syndrome                                    | During sleep                                  |
| 5 m  | ma  | Fulminant myocarditis                                           | During transportation to emergency unit       |
| 5.5 m| fe  | Sudden Infant Death Syndrome                                    | During sleep                                  |
| 7 m  | ma  | Sudden Infant Death Syndrome                                    | During sleep                                  |
| 8 m  | fe  | Sudden Infant Death Syndrome                                    | During sleep                                  |
| 8 m  | ma  | Sudden Infant Death Syndrome                                    | During sleep                                  |
| 3.3 y| ma  | Sudden Unexpected Death Syndrome                                | During sleep                                  |
| 10.1 y| ma | Anomalous origin left coronary artery                           | During exercise                                |
| 12.3 y| ma | Arrhythmogenic right ventricular cardiomyopathy                 | At home                                       |
| 13.7 y| ma | Anomalous origin left coronary artery                           | During exercise                                |
| 15 y | fe  | Sudden Unexpected Death in Epilepsy                             | During sleep                                  |
| 15.6 y| ma | Sudden Unexpected Death in Epilepsy                             | During sleep                                  |
| 15.7 y| fe | Infectious endocarditis (of artificial pulmonic valve)          | During transportation to emergency unit       |
| 17.2 y| fe | Ruptured aneurysm of ascending aorta with hemopericardium       | Found death next to the private pool          |
| 17.8 y| ma | Hypertrophic cardiomyopathy with myocardial disarray            | Drowned                                       |
| 17.9 y| fe | Myocardial infarction due to unknown cause                       | Found death next to her bike                  |

d=days, m=months, y=years, ma=male fe=female
### Supplemental table II: Non-cardiovascular causes of death

| Age | Sex | Cause of death                                      | Circumstances of death |
|-----|-----|-----------------------------------------------------|-------------------------|
| 1   | fe  | Pulmonary hypoplasia                                | At home                 |
| 2   | ma  | Cerebral hypoxia                                    | During sleep            |
| 3   | ma  | Urosepsis                                           | At home                 |
| 4   | ma  | Cerebral hypoxia                                    | During sleep            |
| 5   | ma  | Pneumonia                                           | During sleep            |
| 6   | fe  | Pneumonia                                           | During sleep            |
| 7   | ma  | Waterhouse Friderichsen syndrome                    | At home                 |
| 8   | fe  | Pneumonia                                           | During sleep            |
| 9   | fe  | Pneumonia                                           | During sleep            |
| 10  | ma  | Pneumonia                                           | During sleep            |
| 11  | fe  | Metabolic disorder                                  | During sleep            |
| 12  | ma  | Hypovolemic shock due to purpura fulminans          | At home                 |
| 13  | ma  | Strangulation of the bowel with ischemia            | At home                 |
| 14  | ma  | Pneumonia                                           | During sleep            |
| 15  | ma  | Pneumonia and acute gastroenteritis with hypovolemia| At home                 |
| 16  | fe  | Pneumonia                                           | In the hospital         |
| 17  | ma  | Severe hypovolemia due to acute tubular damage      | During sleep            |
| 18  | fe  | Pneumonia                                           | During sleep            |
| 19  | y   | Strangulation of the bowel with ischemia            | During sleep            |
| 20  | ma  | Pneumonia                                           | During sleep            |
| 21  | ma  | Intussusception due to Meckel’s diverticulum        | At home                 |
| 22  | fe  | Strangulation of the bowel with ischemia            | During sleep            |
| 23  | ma  | Brain herniation due to ventriculoperitoneal shunt dysfunction | During sleep |
| 24  | ma  | Pneumonia                                           | In the hospital         |
| 25  | ma  | Diabetic ketoacidosis                               | During sleep            |
| 26  | fe  | Pneumonia                                           | During sleep            |
| 27  | ma  | Pulmonary hemorrhage                                | During sleep            |
| 28  | ma  | Thyrotoxicosis                                      | During sleep            |
| 29  | fe  | Acute cerebral edema with aqueduct anomaly          | During sleep            |

d=days, m=months, y=years, ma=male fe=female
References

1. Aretz HT. Myocarditis: the Dallas criteria. Human pathology 1987;18:619-24.

2. Basso C, Aguilera B, Banner J et al. Guidelines for autopsy investigation of sudden cardiac death: 2017 update from the Association for European Cardiovascular Pathology. Virchows Archiv : an international journal of pathology 2017;471:691-705.

3. Bajanowski T, Ortmann C, Teige K et al. Pathological changes of the heart in sudden infant death. International journal of legal medicine 2003;117:193-203.

4. Papadakis M, Raju H, Behr ER et al. Sudden cardiac death with autopsy findings of uncertain significance: potential for erroneous interpretation. Circulation Arrhythmia and electrophysiology 2013;6:588-96.