1 **Abnormal muscularization of intra acinar pulmonary arteries in two cases presenting as**
2 **Sudden Infant Death (SIDS).**

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21

22 **ABSTRACT**

23 Abnormal muscularization of acinar arteries is the hallmark of persistent pulmonary
24 hypertension of newborn (PPHN), an uncommon disease with high rate of morbidity and
25 mortality. PPHN presents with signs of respiratory distress immediately following birth. We
26 herein report two cases presenting as a witnessed sudden unexpected death in the late
27 neonatal period (SUDI), preceded by respiratory deterioration and in whom the presence of
28 abnormal muscularization of the acinar pulmonary arteries was reminiscent of PPHN. The
29 significance of this report is twofold; to increase the awareness amongst pediatricians and
30 pathologists of this feature that can present in some cases of SUDI/Sudden Infant Death
31 Syndrome (SIDS), and to highlight the importance of performing a thorough autopsy in order
32 to identify the abnormality.

33 ***Keywords:*** *acinar arteries, autopsy, persistent pulmonary hypertension of newborn, SIDS,*
34 *sudden unexpected death in infancy.*

35

36 INTRODUCTION

37 Persistent pulmonary hypertension of newborn (PPHN) is a severe and well-established
38 pulmonary disorder characterized by a failure of the pulmonary circulation to successfully
39 adapt from fetal to extra-uterine life. It has an incidence rate of about 2 per 1000 live birth
40 (1). Affected newborns are typically term or late preterm and commonly develop symptoms
41 of respiratory distress and severe hypoxemia almost always immediately after birth (2-5).
42 Clinically, neonates with PPHN have elevated pulmonary artery pressure with evidence of
43 right-to left shunt and invariably require supplementary oxygenation and mechanical
44 ventilation. Without proper diagnosis and prompt treatment, the mortality rate of PPHN is
45 high and survivors of PPHN carry with them various long-term functional impairments (4).

46 The histological hallmark of PPHN is the muscularization of the small pulmonary arteries
47 with abnormal extension of vascular smooth muscle into the small, normally non-
48 muscularized intra-acinar arteries (4).

49 We herein report two cases of sudden unexpected death in infancy (SUDI) presenting in the
50 late neonatal period in whom the presence of muscularization of intra-acinar arteries in the
51 lung was reminiscent of the histological features of PPHN. Each case underwent a coronial
52 autopsy examination at our institution as part of the overall investigation into the sudden
53 unexpected death of an infant (SUDI) (6). Whist the first case had non-suspicious nature of
54 death circumstances; the second one was initially related to an allegation of physical abuse.

55

56 CASE REPORT

57 Case 1

58 A 4 weeks-old female child presented to the Emergency Department of a district hospital
59 with a two- week history of increasingly smaller volume of feeds associated with

60 episodic non-projectile vomiting. Two days prior, she became increasingly
61 breathlessness with grunting. At the Emergency Department, she appeared unwell with
62 shortness of breath and tachycardia with a heart rate of 170 beats per minute. On
63 further inspection, she was noticed to have subcostal recession and intermittent
64 grunting which, upon further enquiry, had been present for few days but not known by
65 the parent to be abnormal. Her skin was mottled in appearance and the extremities
66 were cold.

67 There were no signs of rashes, injuries or any marks found on her body. Her respiratory
68 rate continued to increase with increased grunting until a subsequent cardiac arrest. She
69 was pronounced dead following unsuccessful active resuscitation.

70 Her antenatal history was unremarkable. She was born through a Cesarean section at 38
71 weeks of gestation for maternal gestational diabetes. She was apparently well without
72 any health or growth concerns. She has never been admitted to hospital since being
73 discharged after birth.

74

75 A coronial post-mortem examination was requested by the local coroner as part of the
76 overall investigation into SUDI. At autopsy, she appeared well-cared for with neither
77 external dysmorphism nor suspicious injuries detected over the body. Her weight was
78 3880 grams (25th to 50th percentile). The rest of her developmental parameters were
79 appropriate for the age. The internal examination revealed bilateral pleural effusion and
80 ascites of 30 ml. There were few surface petechiae seen over of the thymus and
81 pericardium. The heart appeared enlarged but with no structural abnormality. See Table 1.
82 The ductus and the foramen ovale were closed. The lungs were congested and edematous.
83 The presence of bilateral pleural effusions, ascites, enlarged heart with dilated right
84 atrioventricular chambers was thought to be secondary to acute heart failure (the

ventricular wall thickness was not available in the post mortem report). The examination of the rest of the internal organs was unremarkable.

Case 2

A healthy male neonate was born at term naturally after a normal pregnancy. He was the first child in the family and had no health concerns since birth. On the 12th day of his life, whilst on a journey back home from a pre-arranged hearing test, he appeared to be struggling to breathe and off-color. His condition was progressively worsened and required admission to a nearby hospital. He continued to deteriorate and subsequently arrested and required active resuscitation. Despite efforts at resuscitation, he succumbed to his illness on the same day of hospital admission. Initial examination performed at the Emergency Department as part of overall sudden unexpected death investigation revealed unexplained swelling at the back of head that raised the suspicion of non-accidental injury causing or contributing to death. A forensic autopsy was subsequently arranged , jointly performed by both pediatric and forensic pathologists.

At post-mortem examination, he appeared as a well-nourished and normally formed neonate. His weight of 3655 gram (25-50th centile) and relevant external parameters were appropriate for his age. There were generalized subcutaneous edema that was more prominent over both eye lids and scrotum. There were no injuries detected over the head, trunk and all four limbs. Full skeletal x-rays revealed normal bone remodeling with no bony abnormalities or fractures of either remote or recent in nature.

Internally, the so-called suspicious head swelling appeared as a late-resolving cephalo-hematoma in association with mild biparietal sub scalp edema, which was thought to be

110 further complicated by a perimortem coagulopathy. There were numerous petechiae on
111 the surface of the thymus, heart and lungs. The pericardium contained 22 ml of straw-
112 colored pericardial effusion. The heart was pale, enlarged and with a globous shape. It
113 was structurally normal but the cavities appeared dilated (the ventricular wall thickness
114 was not available in the post mortem report). The foramen ovale and the ductus
115 arteriosus were probe patent. The lungs were increased in weight and showed
116 congested parenchyma. See Table 1.

117 There were submucosal hemorrhages in the upper third of the esophagus with focal bilateral
118 adrenal hemorrhage. The abdomen contained moderate amount of ascites. The liver was
119 within normal weight range but the spleen was enlarged. The associated coagulopathic
120 features in this patient was most likely secondary to deranged liver functions.

121

122 **Histopathological findings and cause of death**

123 In both cases, the histological examination of the lungs showed largely intact bronchial
124 and alveolar architecture except for marked congestion. The pulmonary vasculature was
125 abnormal at the level of intra-acinar arteries (Fig 1). These showed marked thickening of
126 the walls due to persistence of the smooth muscle, intimal proliferation and excess of
127 perivascular collagenous tissue (Fig 2) These changes were highlighted with elastic stain
128 and immunohistochemistry against alpha smooth muscle actin. A muscle coat was
129 present in the pre-capillary arterioles, within the alveolar unit, which under normal
130 development should not persist at this level. There were areas with prominent
131 lymphangiectasis with focal hemosiderosis in Case 1 and bilateral diffuse intra-alveolar
132 hemorrhage and dilated, congested veins in Case 2.

133

134 Bronchial arteries and bronchioles were patent and there was no evidence of

135 misalignment or alveolar capillary dysplasia, confirmed with CD34 immunostaining (not
136 shown). There were no plexiform lesions or occlusion of the bronchial arteries. There
137 was no evidence of an acute or chronic infection, veno-occlusive disease or infarction.
138 Sections of the liver in Case 2 showed venous congestion with presence of extramedullary
139 hematopoiesis, which was associated with presence of nucleated red cells precursors in the
140 peripheral blood, in keeping with a degree of chronic hypoxia.
141 Ancillary investigations performed in both cases which included metabolic, microbiology
142 and toxicology analyses were unremarkable. In none of the cases a cause for secondary
143 pulmonary hypertensive changes was identified. None of the cases had evidence of
144 hypoxic ischemic encephalopathy, meconium or other aspiration.

145

146 **DISCUSSION**

147 In utero, the pulmonary blood flow is low due to a high pulmonary vascular resistance and
148 the availability of shunts through the foramen ovale and the ductus arteriosus. This allows
149 blood to bypass the pulmonary vascular bed. Following the first breath at birth, lung
150 inflation and oxygenation lead to a dramatic fall in the pulmonary vascular resistance. In
151 PPHN, such normal transition does not materialize so that pulmonary vascular resistance
152 remains high. This results in diminished pulmonary blood flow and consequent hypoxemia
153 (3, 4). PPHN is clinically suspected in neonates who present with symptoms and a signs of
154 respiratory distress immediately after birth. It is a difficult and challenging condition to
155 manage clinically and up until recently the mortality rate remains high (6,7). Although the
156 clinical presentation in our cases did not match that of PPHN, the prominent medial
157 thickness of the small arteries was so prominent in our cases and the fact that the witnessed
158 death was preceded by prominent respiratory symptoms was puzzling.

159 An initial study from Naeye (8) described a group of SIDS babies that had 1.6 times as much
160 muscle in their small pulmonary arteries as controls. According to the author (8), the excess
161 of muscle was due to hypertrophy in two third of the cases, whilst it was attributed to
162 hyperplasia of smooth-muscle fibres in the remaining cases. The author's (8) hypothesized
163 that this structural change is the result of chronic alveolar hypoxia. Williams et al (9) study
164 on 15 consecutive SIDS and controls showed an extension of muscle in arteries not usually
165 muscularized, as well as an increase in the percentage of medial wall thickness in some SIDS
166 participants; therefore corroborating Naeye's results (8). Krous et al (10) retrospectively
167 compared the relative medial thickness in alveolar wall arteries, pre-acinar and intra-acinar
168 arteries as well as the demographic, clinical and pathological features in 19 SIDS cases with
169 19 age-matched controls. All cases and controls in their study (10) were >28 days of age,
170 whilst our cases were 12 and 28 days old. With reference to the intra-acinar arteries
171 morphometry, Krous et al (10) showed that age was inversely correlated to the relative
172 medial thickness for small intra- acinar arteries ($p=.018$) and that increasing relative medial
173 thickness of pre-acinar and intra-acinar arteries correlated with increasing relative medial
174 thickness of alveolar wall arteries, suggesting that increasing muscularization is seen in all
175 categories of vessels with a stimulus sufficient to produce it in alveolar wall arteries.
176 Although SIDS and control groups in Krous et al study (10) did not differ for known clinical
177 risk factors that would potentially expose them to hypoxia, the increased thickness of
178 alveolar wall arteries had significantly more males and premature birth than the other
179 groups. Interestingly, the heart's weight in SIDS cases from Krous' study (10) was normal, a
180 significant difference with our 2 cases, where it was above the expected for the age (see
181 Table 1).

182 In an earlier study, Krous et al (11) had found no difference of either increased relative
183 medial thickness or peripheral extension of medial smooth muscle in the small pulmonary
184 arteries (including intra-acinar arteries) between 87 SIDS cases and 17 controls.

185 In the post -natal period the pulmonary circulation exhibits a much lower vascular resistance
186 than the systemic one, and responds to stimuli such as partial oxygen pressure and
187 alterations in blood differently. Hypoxemia dilates the systemic circulation, whereas the
188 opposite is observed in the pulmonary arteries (12). The maintenance of high vascular
189 resistance in the fetus is related to the pulmonary vasculature, reduced oxygen tension,
190 reduced endothelial production of vasodilators (such as nitric oxide, prostacyclin and
191 endothelial growth factor) and increased production of vasoconstrictive prostaglandins (12).
192 The development of excessive musculature in the intra-acinar vascular wall caused by
193 remodeling has a crucial role in vasoconstriction and the increase in vascular resistance.
194 Muscularization of normally non-muscularized peripheral vessels is the result of the release
195 of endothelial vasoconstrictors, growth factors, matrix proteins and adhesion molecules as a
196 cellular response to hypoxia (10, 13)

197 Although our cases fell under the spectrum of Sudden Unexpected Death in Infancy (SUDI),
198 there shared some unusual findings: both died in hospital after a short history of
199 unexplained respiratory deterioration: case 1 had been increasingly breathlessness with
200 grunting for two days, and then presenting with shortness of breath and tachycardia and
201 subcostal recession before arrest; case 2 suddenly struggled to breathe and died shortly
202 after admission. Hypoxia-induced smooth muscle proliferation in pulmonary arteries
203 requires several days (10), so at first sight it seems unlikely that the short lived respiratory
204 symptoms in our cases had any influence in the muscularization of the intra-acinar arteries.

205 However, on further questioning to the parents of Case 1, they had noticed that she had
206 developed subcostal recession and intermittent grunting for few days (but not known this to
207 be abnormal). In addition, the liver in Case 2 showed extramedullary hematopoiesis and
208 there were numerous nucleated red cells in the peripheral blood. This later feature is a
209 known marker of hypoxia and hypoxic mode of death (14,15). In consequence, it can be
210 hypothesized that both our cases had been exposed to a degree of chronic hypoxia.

211 The findings in our two cases are in keeping with those of Naeye (8) and Williams (9) and
212 discrepant with the earlier paper from Krous et al (11) although not significantly with the
213 more recent study from this group (10). The discrepancy could be related to substantial
214 differences between Krous et al (10,11) study population and our 2 cases; mainly: SIDS cases
215 and controls in their study were > 28 days old (our 2 cases were 12 days and 28 days old);
216 the heart's weight in their cases was normal (whilst it was increased in our 2 cases), and their
217 cases had no evidence of chronic hypoxia or respiratory deterioration before death (whilst it
218 was a relevant feature in our cases).

219 The diagnosis of SUDI/Sudden Infant Death Syndrome (SIDS) is a diagnosis of exclusion. In
220 our institution, these cases are thoroughly investigated, with review of the circumstances of
221 death and analysis of all relevant laboratory investigations which included radiology,
222 toxicology, microbiology, cytogenetics and metabolic analyses (6). The autopsy of the first
223 case was jointly performed by both pediatric and forensic pathologists as there was an
224 allegation of non-accidental injury.

225 In summary, we describe two cases presenting as sudden unexpected neonatal death /SIDS
226 in which the abnormal pulmonary vasculature was akin to that of PPHN.

227 Both cases showed respiratory deterioration before the witnessed SUDI and presented
228 muscularization of the small pulmonary arteries with abnormal extension of vascular smooth
229 muscle into the small, normally non-muscularized intra-acinar arteries (4). A study from
230 Ohara et al (16) suggested that in PPHN these pathological findings may represent a
231 pulmonary vascular bed that has been remodeled by the development of the muscular layer
232 in utero. Thus, these abnormalities could have been the result of a variety of prenatal
233 insults, including increased sensitivity to intrauterine hypoxia or stress leading to chronic
234 vasoconstriction and secondary remodeling of the pulmonary circulation (16).

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236 Further investigations are required in order to through address a potential role of these
237 vascular abnormalities in some cases of SUDI/SIDS.

238

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286 Figures

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288 Figure 1: Case 1: a. Muscularization of intra acinar artery (Trichrome x 60) and b: normal
289 age- matched control E: endothelial proliferation (trichrome x 60) and c: Perls stain showing
290 prominent hemosiderin-laden macrophages filling the alveolar spaces indicating previous
291 lung hemorrhage (x20).

292

293 Figure 2: Duplication of elastic lamina (Elastic van Giesson x40)

294

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Table 1: Symptoms, heart and lung weights in case 1 and case 2

Case	Age	Symptoms	Heart weigh (expected)	Lungs weigh (expected)	Lung/body ratio
1	28 days	2 weeks less feeding, vomits. 2 days' breathlessness, grunting. Shortness of breath, tachycardia	36.45 g (21 ± 5)	87.97 (64±27)	0.02
2	12 days	Suddenly struggling to breathe. Respiratory arrest after admission	36.31 g (23±7)	98.68 (64±21)	0.02