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Ocular Findings in Pediatric Deaths Under 2 Years of Age (1994–2004)

ABSTRACT: Our purpose is to highlight novel ocular findings of 102 forensic pediatric cases under 2 years of age who die suddenly. Forensic information, grossing, and microscopic eye protocol was followed. The most common diagnosis was Sudden Infant Death Syndrome (SIDS) (57/102). Novel cytoid bodies were present in the retina of 72/102 cases and they were located predominantly 90% (65/72) at the anterior part of the retina ($p < 0.001$). Of the SIDS cases, 85% (47/57) showed the presence of cytoid bodies, and among all diagnosis, SIDS was the most associated with cytoid bodies ($p = 0.003$). A second observation was extramedullary hematopoiesis (EMH) identified in 35/102 cases and 22 of the 57 SIDS cases. The most frequent EMH location was the choroids (29/35). This study is the first to demonstrate the presence of cytoid bodies and extramedullary hematopoiesis in the retinas of SIDS cases and children who die suddenly from other causes.

KEYWORDS: forensic science, pediatric pathology, sudden infant death syndrome, cytoid bodies, extramedullary hematopoiesis, forensic retinal findings

The investigation of pediatric deaths is considered one of the most difficult areas in forensic pathology (1,2), due to the small stature, different biology, and increased vulnerability of children to abuse. The purpose of this study was to describe ocular findings in children under 2 years of age who die suddenly.

Each year in the United States, more than 4500 infants die suddenly of no obvious cause (Sudden Unexpected Death Syndrome, SUDS). Half of these sudden, unexplained deaths are due to sudden infant death syndrome (SIDS), the leading cause of SUDS and of all deaths among infants aged 1–12 months (3). Worldwide, in countries where unexpected infant deaths are diagnosed as SIDS only after postmortem examination, the death rates from SIDS (20–100/100,000 live births) are comparable to death rates in the United States (77/100,000 live births) (4).

Sudden Infant Death Syndrome (SIDS) is defined as the sudden death of an infant less than 1 year of age which remains unexplained after a thorough case investigation, including performance of a complete autopsy with negative ancillary studies, examination of the death scene, and review of the clinical history including the obstetrical history of the mother, the birth history, and the neonatal history of the decedent child (5,6). Recent panel reviews have modified this definition trying to accommodate new understanding of SIDS and sudden infant deaths, subcategorizing SIDS cases on the basis of specific epidemiologic features including three groups of age (0–21 days, 3 weeks to 9 months, and 9 months to 1 year), and the amount of information available (7,8). SIDS is the leading cause of infant death beyond the neonatal period, mostly between 1 month and 6 months. Although the etiology remains unknown, many factors have been previously associated with the diagnosis. However, recent literature addresses the issues of prone sleeping position, soft sleep surfaces, loose bedding, overheating, smoking, bed sharing, and genetic and metabolic defects (9). Once all of these factors have been controlled, there still remain a large proportion of children who fit the diagnostic entity of SIDS. Male sex, SIDS in a prior sibling, and previous respiratory infection remain independent factors and are permissible in a child who dies of SIDS. A "triple risk" model for SIDS has been proposed: (i) a vulnerable infant, (ii) a critical developmental period in homeostatic control, and (iii) an exogenous stressor (4,10).

Differential diagnoses of sudden death include not only SIDS but also Shaken Baby/Impact Syndrome (SBS) (11), subtle accidents, asphyxias, traumatic child abuse, previously undiagnosed natural disease, and generalized trauma. Retinal examination and findings are a key part of the investigation specifically identifying areas of retinal hemorrhage in those children in whom inflicted injury is suspected. SUDS is defined as sudden death of a child who does not meet the strict diagnostic criteria for SIDS. In many cases of SUDS, a pathologist can identify a cause of death, after a careful review of the circumstances of death and a complete autopsy (12).

Early during the study while the initial focus was to review the ocular findings in children who die suddenly, with the intent to identify features that would allow discrimination between inflicted injury and natural diseases including SIDS, it became apparent that there were interesting new findings not previously identified in the children who died suddenly.

As far as background is concerned, the Regional Forensic Pathology Unit at Hamilton Health Sciences is part of a provincial death investigation system for the Province of Ontario under the direction of the Chief Coroner for the Province. Hamilton Health Sciences is an academic center and the Forensic Unit services a complex community of 2.5 million people covering a large geographical area with a diverse cross section of environments including urban, rural, and recreational areas and also includes farming, heavy industry, light industry, and typical urban services. With regard to pediatric death investigations, it has been the long established practice that all pediatric deaths under 2 years of age have been referred for death investigation in one of four forensic units in the province, with Hamilton Health Sciences being one of the four recognized units. It is staffed by fully trained forensic pathologists

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who practice exclusively forensic pathology. Furthermore, all deaths of children under 2 years of age undergo detailed review by a multidisciplinary group of forensic pathologists, pediatricians, coroners, police, pediatric pathologists, Crown attorneys, and children's aid. Additional contribution of other medical, legal and child abuse subspecialists occurs on an *ad hoc* basis as determined by the individual case needs. This multidisciplinary group reviews all information about a case to ensure accuracy of diagnoses and adherence to internationally accepted criteria for SIDS and other disorders. All cases in this study in which the diagnosis was SIDS met the internationally accepted diagnostic criteria.

Methods

The files of the death investigation of children under 2 years old were selected from the master files of the Gordon V. Terrance Regional Forensic Pathology Unit of Hamilton, Ontario, Canada for the period of 11 years (1994–2004). There were in total 102 cases included in the study. Of the 102, 93 were reviewed retrospectively and nine were prospectively collected.

Forensic information on each case, including the autopsy report, was analyzed and data such as age, sex, cause of death, and postmortem intervals of time were obtained. The histology of all of the cases was re-examined including all of the organ systems. Specific examination of the histology of the hematoxylin and eosin stained slides of both eyes including optic nerves and peri-ocular soft tissue was done.

A grossing protocol for eyes was developed according with the literature (13–16), and applied to the nine prospective cases. This protocol included a gross description with measurements of the globe in three dimensions, orientation, fixation, sectioning, photographs, and histologic processing (Table 1). The most important distinction between the historic specimens and the prospectively collected specimens revolved around detailed description of the specimens, and the fixation in alcohol prior to sectioning of the gross specimens. With additional alcohol fixation, the vitreous humor is jelly-like and easy to cut. The new grossing procedure does not alter the retinal attachment to the choroid; but with formalin fixation only, the vitreous humor is watery, and the retina is easily detached with the dissection. The newer protocol allowed easier handling of the eyes, but did not alter the histologic findings (Fig. 1). We found no morphological differences of the cytoids with no difference in characteristics, location of the retinal layer, and no difference in subsequent immunohistochemical staining. The most significant issue identified with the change in the protocol was the ease with which we were able to handle the eyes allowing for easier sectioning without impact on the histology.

A microscopic protocol was created to review systematically each histological component of the eyes of each case, including a detailed description of conjunctiva, cornea, iris and ciliary bodies, anterior and posterior chamber, lens, vitreous humor, sclera, choroid, retina, optic nerve, and periocular tissue. The retina was anatomically divided as anterior (peripheral or closer to the iris) and posterior (central or closer to the optic nerve), with the equator separating anterior from posterior as an arbitrary line dividing the eye in half.

Composition analysis of cytoid bodies was determined with the following special stains (histochemistry and immunohistochemistry) and performed in five cases:

1. Histochemistry included: Trichrome, Bielschovsky, and Congo red stains.

TABLE 1—*Ocular processing protocol.*

1	Remove eyes with attached optic nerves, by an internal approach to the orbits (orbital roof removal).
2	Fix the intact ocular globes with the attached optic nerves in 10% formalin for 48 h.
3	Rinse eyes with tap water for 24 h.
4	Place eyes in 60% ethanol for a minimum of 24 h prior to sectioning.
5	Describe the external ocular features and measure the anterior-posterior, horizontal, and vertical diameters of the ocular globe, length and diameter of the optic nerve, horizontal and vertical diameters of the cornea, and diameter of the pupil.
6	Trans-illuminate the globes before opening and depending on the findings, use a dissecting microscope to examine the gross abnormalities and photograph.
7	Open the eye with a long bladed scalpel in one smooth cutting motion, by holding the globe firmly and with the cornea positioned down toward the cutting block. The standard technique involves creating two horizontal parallel cuts, made approximately 2 mm from each side of the optic nerve. Open the eye in a clean cut from back to front. The plane of section should begin adjacent to the optic nerve and end through the periphery of the cornea.
8	Observe inside of the globe. If necessary, use the dissecting microscope to describe gross abnormalities and photograph.
9	With this technique three sections (one with cornea-lens-optic nerve section and two caps) (Fig. 1) will be produced. Place each section in individual histologic cassettes and place the cassettes in 60% alcohol for processing.
10	Start the histologic processing in the standard routine for tissue after the steps requiring formalin. Hematoxylin and eosin staining is performed in standard fashion.

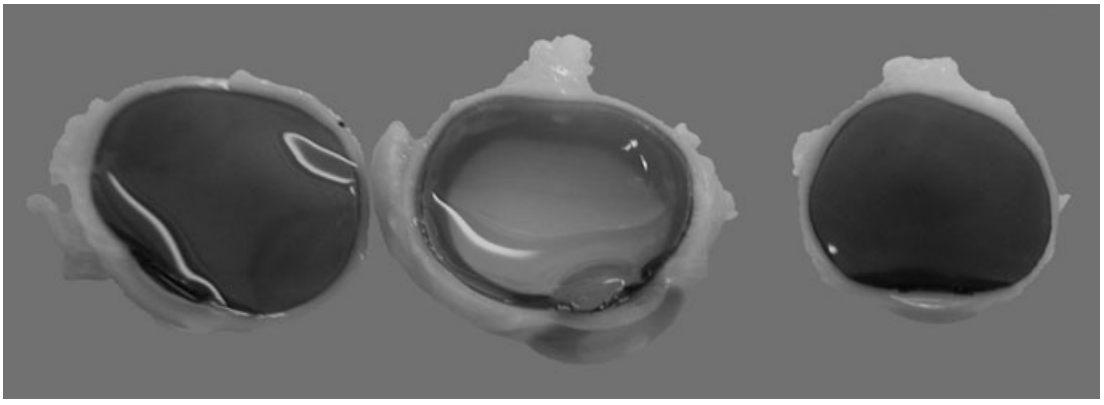

FIG. 1—*Two horizontal parallel sections, and each about 2 mm from the optic nerve, are performed. After that, three ocular fragments are obtained. Notice the jelly-like appearance of the vitreous humor after alcohol fixation, that facilitates the cutting process and avoids distortion of the internal structures, especially the retinal attachment. Internal ocular structures can be examined, described, and photographed with this technique.*

2. Immunohistochemistry included: (i) Monoclonal Mouse Anti: Amyloid precursor protein A4 (Chemicon International, Inc., Temecula, CA), CD56/NCAM Neural cell adhesion molecule (Novocastra Laboratories Ltd., Newcastle on Tyne, U.K.), α-Synuclein (Zymed Laboratories, Inc., South San Francisco, CA), NSE Neuron-specific enolase (DAKO, Carpenteria, CA code No. M 0873), Neurofilament protein (DAKO, code No. M 0762), Vimentin (DakoCytomation, Carpenteria, CA code No. M 7020) and Glycophorin A (DakoCytomation, code No. N 1555); (ii) Polyclonal Rabbit Anti: Synaptophysin (DakoCytomation, code No. A 0010), Prealbumin/Transthyretin (DAKO, code No. A 0002), Calretinin (Zymed Laboratories Inc.), S100 (DakoCytomation, code No. Z 0311),

Extramedullary hematopoiesis was confirmed with the following special immunohistochemical stains and performed in seven cases: (i) Monoclonal Mouse Anti: Glycophorin A (DakoCytomation, code No. N 1555), CD45/LCA Leucocyte Common Antigen (DakoCytomation, code No. M 0701), MAC 387 (DAKOPATTS, code No. M 747); (ii) Polyclonal Rabbit Anti: Myeloperoxidase (DakoCytomation, code No. A 0398).

One case with presence of cytoid bodies was chosen for electron microscopy (EM). This case was a male of 2 months of age and 25 postmortem hours, with diagnosis of SIDS, and presence of cytoid bodies and extramedullary hematopoiesis. By light microscopy, the anterior (peripheral) zone of retina was assessed and an area close to the ora serrata with numerous cytoid bodies was selected. This area was removed from the paraffin block and submitted for EM.

Obtained data was summarized by frequencies, cumulative frequencies, percentages, pie and bar graphs, and cross-tabulation. Categorical variables were compared using chi-square test. *p*-value of less than 0.05 was considered statistically significant.

Results

Our initial study population consisted of 102 children (66 male and 36 female). The majority of the cases, 59 (60.18%), were concentrated between 1 and 6 months of age. The most frequent diagnosis was SIDS (57/102), followed by SUDS (9), head blunt trauma (8), and different forms of positional asphyxia (7) (Fig. 2). The male sex was predominant in the SIDS cases (37/57).

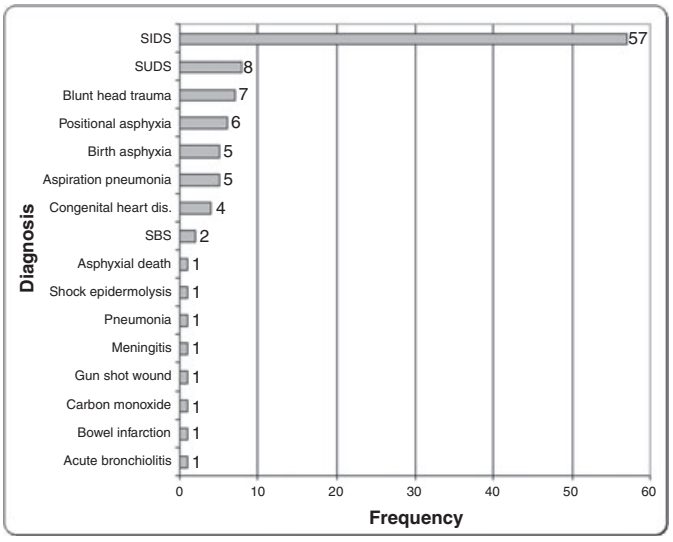

FIG. 2—*Distribution of cases by diagnosis (n = 102). SBS = Shaken baby syndrome, SIDS = Sudden infant death syndrome, SUDS = Sudden unexpected death syndrome.*

Novel cytoid bodies in the retina were identified in 72/102 (70.6%) cases, the majority of which were males (46). Of the 72, 66 cases (91.6%) had cytoid bodies in both eyes.

Cytoid bodies were histologically located at the sub-hyaloid space involving the internal limiting membrane and nerve fiber layer of retina. The sub-hyaloid space is anatomically located below the hyaloid membrane, which is the membrane around the vitreous humor and in contact with the internal limiting membrane (the most internal layer of the retina). Cytoid bodies, on hematoxylin and eosin stain, are rounded to pyriform in shape, with a size variation (5–20 μm), amphophilic color (sometimes more eosinophilic), and smooth to finely granular/homogeneous cytoplasm. They can mimic red blood cells (RBCs); however, the cytoids lack the biconcave disc shape and central pale area typical of RBCs, and have less eosinophilic staining than RBCs (Fig. 3). Depending on the orientation of the section, the cytoid bodies appeared attached to the retina sometimes by a tiny strip of retina or floating in the sub-hyaloid space. Under high power examination (60× or 100×), the cytoids appeared either homogeneously amphophilic or diffusely micro-granular with multiple basophilic spots. Nuclear material was never identified. The cytoplasmic membrane appeared slightly denser and basophilic.

Even though the cytoid bodies were clearly identified by hematoxylin and eosin stains, histochemical and immunohistochemical studies were attempted. Five cases identified were subjected to this staining protocol and all of the cytoid bodies demonstrated the same characteristics. Cytoid bodies stained dark red with trichrome and dark brown with Bielschovsky stains. Congo red was negative. Positive immunohistochemical markers included S100, transthyretin, synuclein, and CD 56. Negative immunohistochemical markers included glycophorin A, amyloid precursor protein, vimentin, synaptophysin, calretinin, nonspecific enolase (NSE), and neurofilament protein. The immunohistochemical characteristics were clearly expressed suggesting that postmortem decomposition was not important. Immunohistochemistry is notoriously sensitive to decomposition with unreliable expression of various markers, seen with the inability of most tissue to stain adequately. This problem

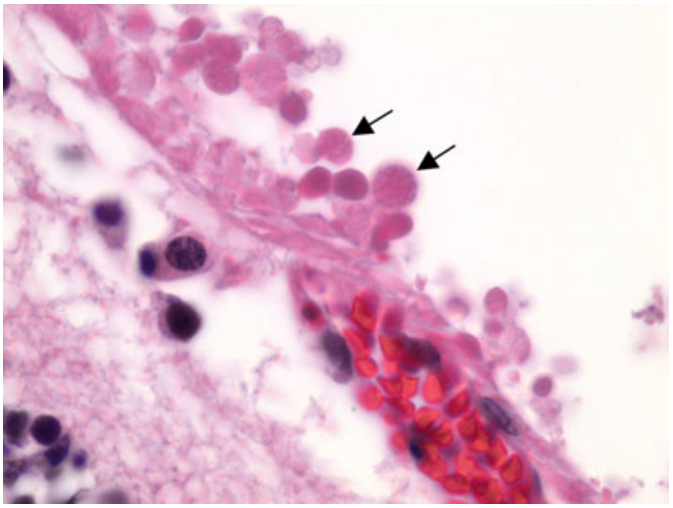

FIG. 3—*Cytoid bodies (arrows). Notice the rounded shape, mild to moderate size variation, amphophilic to eosinophilic color, smooth to granular/homogeneous cytoplasm, and size ≥ a red blood cell. The shape of the cytoid lacks the biconcave shape or central pallor typical of red blood cells, and has a less eosinophilic color than red blood cells. 10× 40× power of hematoxylin and eosin stain.*

was never encountered in the cases subjected to the immunohistochemical panel used in this study.

Because of the nature of forensic cases being always after death, a comparison to surgically excised eyes was not possible. Careful examination of the remainder of other tissues of the decedent children for the presence of decomposition failed to identify that decomposition was a factor. The cytoid bodies were exclusively seen in the anterior part of the retina and there was no increased numbers or difference in location of the cytoid bodies in children who did demonstrate very mild decomposition. Furthermore, there was no statistically significant relationship between the postmortem interval and the presence of cytoid bodies.

If present, cytoid bodies were located predominantly 65/72 (90.2%) at the anterior (peripheral) part of the retina with a significant association ($p < 0.001$) (Fig. 4). Of the SIDS cases, 85% (47/57) showed the presence of cytoid bodies and among all diagnosis, SIDS was the most associated with cytoid bodies ($p = 0.003$) (Fig. 5).

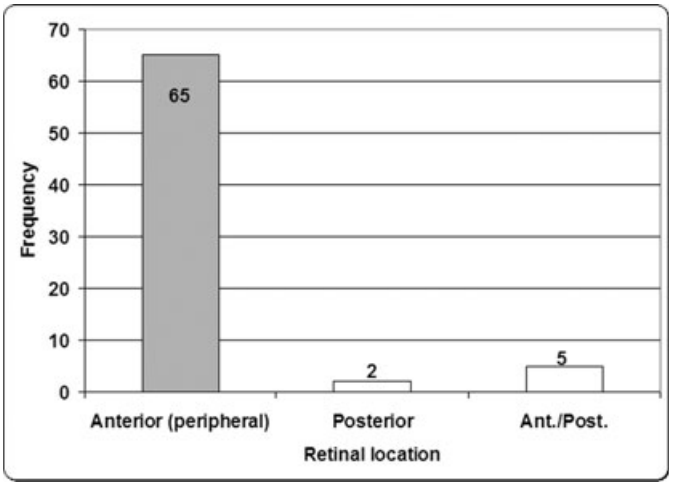

FIG. 4—*Retinal location of cytoid bodies (n = 72). Cytoid bodies in the majority of cases were located at the anterior part of the retina (65). Two cases were located at the posterior part and 5 in both sites.*

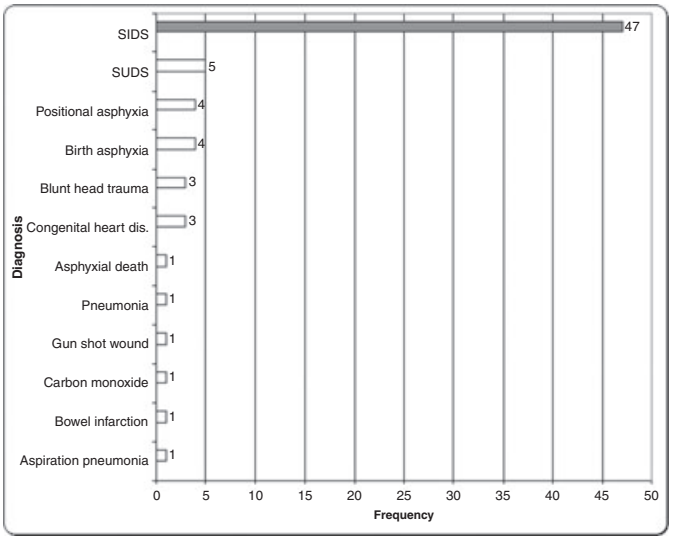

FIG. 5—*Distribution of cases with cytoid bodies by diagnosis (n = 72). SIDS was the most frequent diagnosis with the presence of novel cytoid bodies.*

Retinal involvement was defined arbitrarily by percentage of retina demonstrating cytoid bodies. The majority of cases (52/72) showed ≤25% of retina with cytoid bodies, 15/72 showed 50%, 4/72 showed 75%, and 1/72 showed ≥ 75%. Of the 72 cases with cytoids, 67 had a postmortem interval of time of less than 40 h. In these cases, 49/67 showed ≤25% of retina with cytoid bodies, 13/67 up to 50%, 4/67 up to 75%, and 1/67 ≥ 75%. In the remainder of the cases (5/72), the autopsy was performed at 40, 47, 50, 72, and 94 h after death, and these cases showed retinal cytoid bodies ≤25%, 50%, ≤25%, 50%, and ≤25%, respectively. There was no significant statistical relationship between cytoid bodies and the postmortem interval ($p = 0.77$). Furthermore thirty cases did not show cytoid bodies despite the observation that five of these cases had a postmortem interval of 24 h. This indicates that the cytoids were not a postmortem artifact. Of the 102 cases, 72 underwent CPR, and 51 of these 72 showed cytoid bodies, with no significant association ($p = 0.93$).

The age distribution of the total 57 cases of SIDS was similar to the distribution of SIDS cases reported in the literature (4,5), showing that the majority, 43 cases (75.4%), were between 1 month and 4 months of age.

Cytoid bodies were examined by electron microscopy, and showed rounded shape, lined by a fine membrane, attachment to the nerve fiber layer, and with a cytoplasm containing numerous fragments of membranes, possibly from cellular organelles (Fig. 6). Due to ultra-structural postmortem changes (25 postmortem hours), more specific analysis of the contents of the cytoids could not be ascertained.

A second important finding noted during this study was extramedullary hematopoiesis (EMH) (Fig. 7), which was identified in 35/102 cases, with a sex distribution of 24 male and 11 female. Of the 35, 24 cases (68.5%) showed EMH in both eyes, and 24/35 had coexisting EMH and cytoid bodies. When these 35 cases with EMH were distributed by diagnosis, 22 cases (62.8%) had a diagnosis of SIDS approaching statistical significance ($p = 0.06$). When EMH was combined with cytoid bodies, 16/24 had diagnosis of SIDS. EMH cases (17) were distributed by age and demonstrated eight cases in the first month of age, 15 between 1 and 2 months, 6 between 2 and 3 months, three cases between 3 and 5 months of

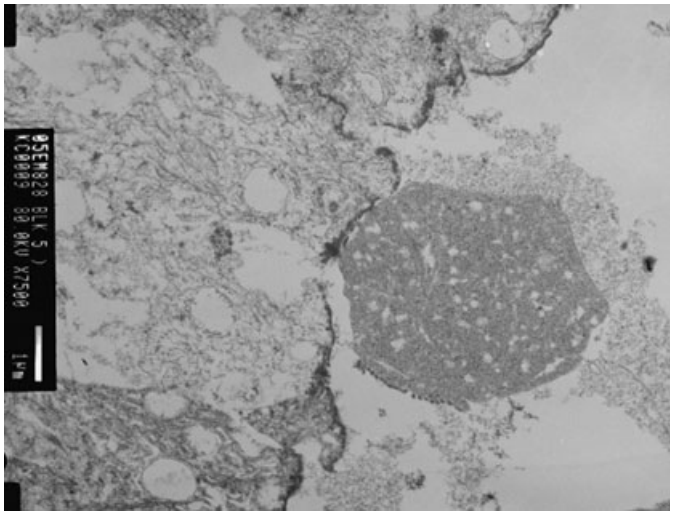

FIG. 6—*Electron microscopy of cytoid bodies located at the subhyaloid space and over the internal limiting membrane. Note the rounded to oval shape and different densities of the cytoplasm with fragmented structures. Internal organelles are not clearly identified due to postmortem autolysis.*

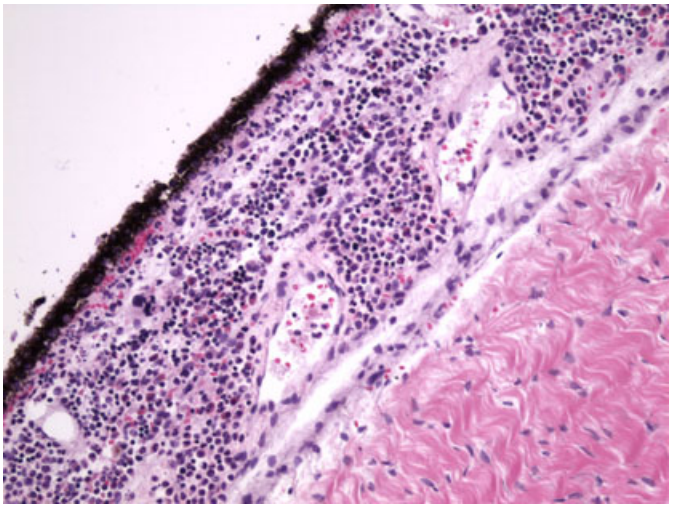

FIG. 7—*This photomicrograph illustrates an excellent example of extramedullary hematopoiesis as was noted in many sections of the cases examined.*

age, and 1 each at 12, 18, and 24 months. There were eight deaths due to head trauma and none of these demonstrated EMH.

EMH was primarily located at the choroid in 82.8% of the cases (29/35). In some instances, the EMH was present in more than one ocular region. The choroid and the sclera were the most frequent combination (11 cases) of location. Rare cases showed EMH around the optic nerve or around blood vessels at the peri-ocular soft tissue. All cases with ocular EMH were reviewed in detail with examination of the histologic slides from other organs in an effort to delineate EMH in other typical locations. Of the 35 cases, six had EMH in the liver, one in the spleen, and one in the thymus.

EMH was readily noted with H&E stains and demonstrated myeloid, erythroid, and lymphoid lines. Some cases had obvious megakaryocytes. Bone marrow immunohistochemical profiles were performed and the typical findings were obtained with positive staining with myeloperoxidase and MAC 387 for the myeloid lines, glycophorin A for the erythroid line, and leucocyte common antigen (LCA/CD45) for the lymphoid lines.

Transretinal hemorrhage was not observed in any of the cases of SIDS.

Discussion

The investigation of deaths of children is always a complicated process which is in constant evolution particularly where understanding of pathophysiologic mechanisms are concerned. We would like to address a few issues as a preface to discussion of the findings of this study. The first is that the diagnosis of SIDS is constantly changing when obscure causes of death are identified to account for a previously documented death of a child, for example the prone sleeping position or death in an unsafe sleeping environment. Prior to inclusion in our study the diagnosis of SIDS was reassessed. This included a review of the history, physical findings, scene information, summary of the review panel and other investigations to ensure that all those remaining with a diagnosis of SIDS would meet the current standards.

We learned that the eye is a very complex anatomic structure and a standardized approach and protocol are necessary for the evaluation of the ocular findings in children who die suddenly. A protocol was developed using various sources in the medical literature as the basis for our approach, combining the gross and microscopic processing recommended in those studies (13–16,18).

Also, it is important to remember that the retina is a delicate nervous tissue, upon which the images of external objects are received. The outer retinal surface is in contact with the choroid and the inner retinal surface is in contact with the hyaloid membrane of the vitreous body. Posteriorly, the retina is continuous with the optic nerve, is gradually diminished in thickness from posterior to anterior, and extends nearly as far as the ciliary body, where the retina appears thinnest and to end in a jagged margin, the ora serrata. The retina consists of an outer-pigmented layer and an inner nervous stratum or retina propia, which consists of multiple layers. The nervous layers of the retina are connected together by a supporting cellular framework, formed by the sustentacular fibers of Muller cells, the ends of which form the internal and external limiting membranes. The nerve fiber layer is composed by unmyelinated ganglion cell axons, which will form the optic nerve. The central artery of the retina and its accompanying vein pierce the optic nerve, and enter the bulb of the eye through the porus opticus. The artery immediately bifurcates into an upper and a lower branch, and each of these again divides into a medial or nasal and a lateral or temporal branch, which at first run between the hyaloid membrane and the nervous layer. The vessels soon enter the latter, and pass forward, dividing dichotomously. From these branches a minute capillary plexus is developed, which does not extend beyond (in depth) the inner nuclear layer. The branches of the central artery do not anastomose with each other—in other words they are terminal arteries, and do not supply blood beyond (in depth) the inner nuclear layer (19). The resultant blood supply to the periphery (anterior retina) is less and the vessels are of small caliber.

This study demonstrated that the observed cytoid bodies of the majority of cases are located at the periphery of the retina (90.2%), where the blood supply is the most limited. To the authors' knowledge, this is the first report that describes cytoid bodies in sudden death in pediatric cases. Our study identified cytoid bodies in 72/102 deaths and the subgroup of SIDS represented the highest incidence (47 cases) with a significant association ($p = 0.003$).

Due to histological similarity with red blood cells, the immunohistochemical profile of the cytoids was determined and compared with RBCs (glycophorin A). Glycophorin A (sialoglycoprotein alpha) is one of two transmembrane proteins exposed on the outer surface of normal human erythrocytes. Furthermore, glycophorin A is expressed during all stages of differentiation, from the normoblast to the mature erythrocyte (20). In our cases, cytoid bodies were completely negative for glycophorin A, so we concluded that they were not of erythroid origin.

Since the cytoids are located in the retina, multiple markers were utilized to further characterize the origin of these structures. Cytoid bodies were consistent positive for synuclein, S100, CD56, and transthyretin.

Synucleins are small proteins (14.5–20 kDa) found expressed in the brain and located in the neuronal cytosol and presynaptic terminals. Recently, there has been much interest in this protein, due to the association with neurodegenerative diseases (Alzheimer, Parkinson, Lewy body dementia) and cancer (breast). Recent studies have shown that all members of the synuclein family are expressed in the retina and optic nerve (21). Additionally, α-synuclein has been implicated in mitochondrial dysfunction and oxidative stress associated with neurodegenerative diseases (22).

CD56, also called N-CAM (neural cell adhesion molecule), appears on early embryonic cells and is important in the formation of cell collectives and their boundaries at sites of morphogenesis.

Later in development it is found on various differentiated tissues and is a major CAM mediating adhesion among neurons and between neurons and muscle (23). CD56 is expressed in a membranous pattern and we observed a similar membranous pattern in our cases.

Transthyretin (prealbumin), one of the T4 binding proteins, is implicated in retinol and amyloid metabolism. The liver represents the main source of transthyretin synthesis, but choroid plexus and retina produce small amounts of this protein. Transthyretin and S100 (an acidic protein of neural cells) were positive in cytoid bodies.

As a result of these positive markers and with the addition of the positive Bielschovsky stain, the cytoid bodies were shown to be of neural retinal origin.

Cytoid bodies have been described by electron microscopy to be formed by swelling of nerve fiber ends accompanied by accumulation and degenerative changes of axoplasmic organelles such as mitochondria, neurofilaments, and dense bodies (1,24–26).

The first report of retinal cytoid bodies was in 1856 (27). Since then, many reports have been written about cytoid bodies associated with different diseases, such as Acquired Immuno-Deficiency Syndrome (AIDS) in association with cytomegalovirus retinitis (25,28), and in diabetic retinopathy (29), hypertensive retinopathy (30), scleroderma (31), septicemia (32), and in animal models with ischemic retinopathy after the intravenous injection of talc (33).

Occlusion of retinal arterioles may produce infarcts of the nerve fiber layer of the retina (axons of the retinal ganglion cell layer populate the nerve fiber layer). Axoplasmic transport in the nerve fiber layer is interrupted at the point of axonal damage, and accumulation of mitochondria at the swollen ends of damaged axons creates the histologic illusion of cells (*cytoid bodies*). Collections of cytoid bodies populate the nerve fiber layer infarct, seen ophthalmoscopically as "cotton-wool spots." Although nerve fiber layer infarcts are described in the context of hypertension, they may be detected in a variety of retinal occlusive vasculopathies. For example, retinal nerve fiber layer infarcts may develop in AIDS patients secondary to a retinal vasculopathy that is similar to the vasculopathy that may develop in the brain in this condition (26).

Multiple reports and animal models of ischemic retinopathy have been hypothesized to account for the production of cytoid bodies (33). Characteristics of cotton-wool spots (histologically, they are accumulations of cytoid bodies) in patients with hypertension and HIV have been demonstrated with optical coherence tomography, as lesions of the nerve fiber layer of the retina, that usually accompany a wide variety of systemic diseases (34). New hypotheses have been offered implicating alterations in axoplasmic transport (orthograde: axoplasmic flow going to optic disc, retrograde: axoplasmic flow going to ganglion cells). Some of these hypotheses propose: acute hypo-perfusion of the central retinal artery, with slow flow along its branches and ischemic gradient affecting progressively more peripheral locations in the inner retina; vasoneural compression of retinal nerve fiber layer; reflux of venous blood through the valveless jugular veins and cavernous sinus to branch retinal venule which is acutely hyperdistended from transmission of transient extreme elevation of the intrathoracic venous pressure to the eye, and doing compression of adjacent axon bundles and obstruction of both orthograde and retrograde axoplasmic transport, with subsequent cytoid body formation (35).

Regardless of the theory of how it develops, the cytoid bodies appear to have resulted from localized ischemia of a sensitive portion of the retina, i.e. the peripheral location as observed in 90.2% of our cases.

While it is remotely possible that the finding of cytoid bodies remains a postmortem artifact, cytoid bodies were rarely identified in children who died due to trauma (four cases) despite a postmortem interval similar to that of the SIDS cases. This would add credence to the conclusion that these findings were not due to decomposition, keeping in mind that with electron microscopy the very earliest of decomposition makes organelles lose their ultrastructural appearance quickly. The investigators tried to utilize an animal model for examining the robustness of the tissue from an ultrastructural perspective but the animal model was not accepted as an equivalent. Other studies have looked at surgical specimens dating back to the late 1800s as previously discussed and these offer no discrepant results from our population with reference to the content and composition of cytoid bodies.

Arguments that cardiopulmonary resuscitation (CPR) may have resulted in retinal vascular distention and cytoid body formation by vasoneural compression was considered. In our study 51/72 cases with cytoid bodies underwent CPR.

EMH in the eyes has been previously described (17). The largest series had 19/122 cases, with 12 in premature and seven in full term infants. Two infants lived up to 67 and 107 days and the EMH was thought to be due to the extreme prematurity of these children. The authors suggest that the choroid may be one of the physiologic sites of hematopoiesis during fetal life, diminishing rapidly and disappearing after birth. Isolated adult cases also have been reported with choroid extramedullary hematopoiesis associated with traumatic globe rupture, penetrating injuries, and enucleation a few days later (36,37). Late enucleation after medical conditions such as trauma, inflammation, congenital anomalies, and retinal detachment have shown that eyes may produce intraocular ossification and hematopoiesis (38).

We report the largest collection of pediatric cases with EMH in eyes (35/102). Hematopoiesis was confirmed by immunohistochemistry for myeloid, erythroid and lymphoid lines. The majority of the cases (21/35) were of the age one to 3 months, and the majority had a SIDS diagnosis (22/35). When EMH was combined with cytoid bodies (25) the most common diagnosis was SIDS (16/24). Extramedullary hematopoeisis may be an artifact of the neonatal period of time; however, the incidence of this finding decreases with age and typically has disappeared within weeks of birth.

This study is the first to demonstrate the presence of extramedullary hematopoiesis and cytoid bodies in the retinas of SIDS children.

The two cases of victims of SBS did not demonstrate EMH or cytoid bodies. SIDS cases did not show trans-retinal hemorrhage in contrast to cases of SBS.

Recently there have been many reports in the forensic literature examining the value of fundoscopic examination of the retina shortly following death (39,40). In our experience and opinion, we find it also equally important to examine the eyes grossly and microscopically. Other novel findings may be encountered and we look forward to other investigators' observations. The observed features that we have described can be easily identified by routine histologic processing and staining with hematoxylin and eosin, without the necessity of immunohistochemistry, allowing wider applications using services available in most forensic units. Furthermore, the cost should be low, requiring only three additional slides per case under most circumstances. While in this study we employed many markers and special stains to determine the nature and origin of the findings, especially the cytoid bodies, this would not be necessary in practice, thereby representing a very cost effective use of resources to result in a significant addition to the understanding of the pathophysiologic mechanisms in play in these unfortunate deaths.

In conclusion, this study demonstrates that cytoid bodies and extramedullary hematopoiesis are frequently identified in children who die suddenly. Since the most common cause of death was SIDS in our study population and that amongst the children with SIDS there was a majority demonstrating these findings, it may introduce the possibility that the common pathway in the pathophysiologic process might be due to subtle subclinical hypoxic injury. Furthermore the hypoxia is likely chronic or recurrent given the presence of EMH since this would not occur as an acute event.

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