Neuropathologic Examination in Sudden Unexpected Deaths in Infancy and Childhood: Recommendations for Highest Diagnostic Yield and Cost-Effectiveness in Forensic Settings

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ABSTRACT

In infants and toddlers (less than four years of age), determination of cause and manner of death often requires a complete autopsy. Few evidence-based guidelines exist regarding optimum nervous system sectioning in this population. Over a six-month interval and using a comprehensive section protocol, we categorized cases having neuropathological findings that were critical (Class A), contributory (Class B), or noncontributory (Class C) to the final cause and manner of death. We further evaluated which sections helped make this determination. Among 53 cases (44 infants, 9 toddlers; 26 girls, 27 boys), Class A neuropathology was noted in nine (16.9%). Seven infants had meningoencephalitis (2/7, 28.6%), craniospinal trauma (3/7, 42.8%), brainstem necrosis suggesting Leigh Disease (1/7, 14.3%), and hydrocephalus in Dandy-Walker malformation (1/7, 14.3%); two toddlers had inflicted craniospinal trauma (2/2, 100%). Class B factors were identified in 11/53 (20.8%), including recent hypoxic-ischemic lesions (2/11, 18.2%), meningitis or dural venous sinus thrombosis in systemic sepsis (2/11, 18.2%), multicystic encephalopathy following peripartum asphyxia (2/11, 18.2%), and microcephaly and delayed myelination (Cri-du-Chat Syndrome) (1/11, 9.09%). Class B also included three toddlers (3/11, 27.2%) with features of hippocampal dysgenesis, two in the setting of febrile seizures. Class C comprised normal brains (3/53, 5.7%), and those with findings of uncertain significance, such as white matter and brainstem gliosis (30/53, 56.6%). The sections most valuable for detection of relevant pathology, and thus recommended for routine sampling, were: 1) bilateral hippocampus; 2) cerebral cortex and leptomeninges; and 3) pons or medulla. *Acad Forensic Pathol.* 2017 7(2): 182-199

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INTRODUCTION

Forensic agencies throughout the world generally aim to follow the mandate, emanating from the original National Institutes of Child Health and Human Development recommendations from 1991 (1), to perform a complete autopsy, scene investigation, toxicologic, and microbiologic studies in cases of sudden unexpected infant death (SUID) and, by extension, sudden unexpected death in childhood (SUDC). While the neuropathologic examination is a key component of the complete autopsy, forensic pathologists vary in their approach to the central nervous system for several reasons: 1) the uneven availability of neuropathologic expertise or consultation; 2) the lack of staff and material support for the special techniques often required for proper brain tissue processing; and 3) variability in the extent of training and experience in analysis of the developing brain.

The most frequently cited published protocol for neuropathologic examination in SUID is that of the California Department of Public Health. In that protocol, the following blocks are considered “mandatory” for microscopic analysis: pontomesencephalic junction, pons, midbrain, hippocampus, frontal lobe, cerebellum, and choroid plexus; basal ganglia sampling is “discretionary” (2). Of course, any macroscopic abnormalities should also be sampled. A similar approach is advocated by the Royal College of Pathologists. In that protocol, the guidelines specify sections of cerebral hemisphere, brainstem, cerebellum, meninges, and spinal cord, as well as dura if hemorrhages are noted, following two to three weeks fixation in formalin (3).

In this study, we sought to determine, using an evidence-based approach, the optimal examination protocol that would be both high-yield for important diagnostic details and also resource-conscious, given the constraints under which many forensic agencies, including our own, must operate.

In this article, we report our analysis of an unselected series of infant and toddler deaths during a six-month interval, during which we extensively sectioned the

nervous system of all cases in a uniform way. We scrutinized two outcomes. The first was whether the neuropathologic examination was critical (Class A); contributory (but non-critical, Class B); or noncontributory (Class C) to the medical examiners’ rendering of cause and manner of death (COD and MOD, respectively). The second measure was which brain sections were most frequently used to detect critical and contributory features.

Based on our findings, we recommend a “minimal” section protocol for histology processing (i.e., slides for review prior to finalization of death certificate). Moreover, arising from our experience with this small series, we also suggest a “stock section” protocol for backup, in cases having more complex or unforeseen features requiring further workup.

METHODS

During a six-month period, we examined all brain specimens from live-born infants (aged up to one year) and toddlers (aged from one year and one day up to four years) received at the Office of the Chief Medical Examiner, New York City (NYC OCME). We included neonates less than one week of age for the sake of completeness and consistency. At our agency, the medical examiners refer all such pediatric cases to the neuropathology service after placing the brain, along with any other related samples (e.g., spinal cord, dura, eyes, cervical vertebral block [vertebrae and paravertebral soft tissues, with spinal cord and nerve roots *in situ*]) in 20% neutral-buffered formalin for a fixation period of one to three weeks. While we recognize that some of the referred cases had a presumed cause and manner of death (e.g., child abuse, drowning), we included all infant and toddler cases as an unselected series in order to apply our uniform approach, as a means of determining the utility of various aspects of examination.

The submitted specimens were then evaluated using a uniform protocol developed by Robert’s Program on Sudden Unexpected Death in Pediatrics (4), based at Boston Children’s Hospital in Boston, Massachusetts (5). The histologic section protocol is comprehensive, identifying 17 specific neuroanatomic sites, including

cerebral hemispheres, brainstem, cerebellum, and spinal cord, for standard processing and paraffin-embedding (**Table 1**). Eyes and/or optic nerves and cervical cord/vertebral blocks with nerve roots were obtained by the referring medical examiner and processed according to published guidelines (6, 7); these samples, however, were not included in the analysis of diagnostic yield.

Hematoxylin and eosin stains were applied using standard laboratory techniques. Perl's stain for iron was done in a subset of cases (usually those in which trauma was suspected). Immunostains for various an-

tigens (such as CD45 [leukocyte common antigen], amyloid precursor protein [APP], or macrophage markers [CD68]) were obtained in selected cases.

The slides were reviewed by one of us (RDF) and one or more of the other authors. Additional data (macroscopic photographs, scene investigations, toxicology, microbiology, radiography, forensic anthropology, metabolic, and/or genetic reports) were reviewed. A final neuropathologic diagnosis was made available to the medical examiner to use as needed in their determination of cause and manner of death.

Table 1: Section Protocol for Neuropathologic Examination of Sudden Unexpected Infant Death and Sudden Unexpected Death in Childhood, and Diagnostic Yield of Particular Histologic Sections With Regard to Diagnostic Class A or B

Section	Cases Having Section (% of 53 Total)	Contribution of Section to Class A or B (% of 20)
1. Posterior cingulate gyrus, including corpus callosum	47 (88.7%)	7 (35.0%)
2. Right insula and superior temporal gyrus	45 (84.9%)	1 (5.00%)
3. Left insula and superior temporal gyrus	42 (79.2%)	1 (5.00%)
4. Amygdala	41 (77.4%)	3 (15.0%)
5. Right hippocampus at level of lateral geniculate nucleus	49 (92.5%)	10 (50.0%)
6. Left hippocampus at level of lateral geniculate nucleus	47 (88.7%)	9 (45.0%)
7. Basal ganglia (caudate, internal capsule, globus pallidus, putamen)	51 (96.2%)	8 (40.0%)
8. Hypothalamus (mamillary bodies)	43 (81.1%)	4 (20.0%)
9. Thalamus, posterior, including internal capsule	50 (94.3%)	5 (25.0%)
10. Calcarine cortex	48 (90.6%)	7 (35.0%)
11. Parietal cortex and white matter at atrium of lateral ventricle	49 (92.5%)	6 (30.0%)
12. Midbrain	45 (84.9%)	9 (45.0%)
13. Pons	51 (96.2%)	9 (45.0%)
14. Medulla (rostral and caudal)	51 (96.2%)	12 (60.0%)
15. Cerebellar vermis	43 (81.1%)	0
16. Cerebellar hemisphere including dentate nucleus	52 (98.1%)	4 (20.0%)
17. Spinal cord	44 (83.0%)	7 (35.0%)
18. Other*		
Frontal or other cortex, not otherwise specified	12 (22.6%)	3 (15.0%)
Eyes and/or optic nerves*	10 (18.9%)	NA
Cervical nerve roots, bone, soft tissue*	3 (5.7%)	NA
Dura	21 (39.6%)	7 (35.0%)

NA - Not analyzed

* Sections not part of Robert's Program protocol.

The significance of the neuropathologic findings was qualitatively determined by consensus of all authors as the number and proportion of cases in which the neuropathologic diagnosis was 1) critical (Class A); 2) contributory (Class B); or 3) noncontributory (Class C) to assigning the final cause and manner of death. Class A findings were defined as those primarily or independently responsible for the death of the child (e.g., severe cerebral or spinal trauma, meningitis). Class B findings were considered to be those that did not primarily cause the child's demise, but represented significant lesions (e.g., dural venous sinus thrombosis in sepsis, multicystic encephalopathy from birth asphyxia). Class C cases had no neuropathology or abnormalities insufficient to explain or otherwise predispose to death (e.g., diffuse white matter or brainstem gliosis); we acknowledge that in these cases, the neuropathologic examination ultimately does contribute to determination of COD and MOD by excluding significant lesions of the nervous system. Notation was also made of additional particular findings of uncertain significance, potentially meriting focused research; these features were broadly labeled "possible microdysgenesis" (8-14) and are currently under separate analysis.

For purposes of this study, examples of some Class A and B diagnoses are illustrated in the Results. For some Class C diagnoses, the following features were used. For diffuse white matter gliosis, the presence of two or more astrocytes with hypertrophic cytoplasm per high-power field (x400) was considered sufficient. Care was taken not to overcall myelination glia (**Image 1A**), characterized by small, dense nuclei, and dense globoid pink cytoplasm, usually no larger than the size of the glial nucleus. Reactive (hypertrophic) astrocytes, by contrast, had pink cytoplasm with stellate processes and nuclei with more open chromatin. For an interpretation of brainstem gliosis (**Image 1B**), reactive astrocytes (as just defined) could be present in the inferior olivary nucleus neuropil, intralaminar pontine nuclei, median raphe, or, more rarely, other sites. Periventricular leukomalacia required the presence of focal necrosis (axonal spheroids in the acute setting) (**Image 1C**); macrophages and/or cavitation are seen in later stages of evolution (not illustrated).

Acute neuronal ischemia (ANI) was noted when neuronal nuclei were condensed and angulated, and the cytoplasm was hypereosinophilic; sometimes the nuclei were fragmented (apoptotic) (**Image 1D**); usually the neuropil was vacuolated as well. Care was taken not to overcall cells with just pyknotic nuclei. When ANI was diffusely present, the term hypoxic-ischemic encephalopathy (HIE) was used. Multicystic encephalopathy applied to multifocal (or subtotal) loss of cerebral gray and white matter, with cavitation and gliosis (i.e., HIE with chronic or organized features). Ulegyria refers to the loss of cortical volume at depths of sulci, with relative sparing of crests. Laminar necrosis was identified when segments of cortical plate showed acute ischemia or post-ischemic loss. Hypoxic-ischemic myelopathy referred to central gray matter necrosis in the spinal cord.

Hemorrhagic lesions (subdural, subarachnoid, epidural, germinal matrix, ocular; not illustrated) were called "acute" if intact red cells were seen in the specified site; "subacute" if scattered macrophages or occasional spindle cells (fibroblasts) were infiltrating; and "chronic or old" when hemosiderin, macrophages, fibroblasts, and fine neovasculature were seen.

Meningoencephalitis required inflammatory cells within the leptomeninges and cortical parenchyma, the latter characterized by microglial nodules and/or neuronophagia (see Results). Focal slight inflammation in any site was considered nonspecific, and not used for diagnosis.

Hippocampal dysgenesis was determined based primarily on dispersal and bilamination of the dentate gyrus, as well as macroscopic asymmetry of the hippocampal formations, as described by Kinney and colleagues (7, 10) (see Results).

RESULTS

The demographic characteristics, causes and manners of death, and neuropathologic findings are shown in **Table 2**.

Image 1: Some common histologic features seen in the cohort (see also **Table 2**). **A)** Myelination glia in cerebellar white matter have dense, eosinophilic, inclusion-like globular cytoplasm, of lesser volume than the oligodendroglial nucleus (arrows) (Case 26) (H&E, x600). These are not pathologic. **B)** By contrast, reactive astrocytes in the inferior olivary nucleus have eosinophilic star-like processes (arrows), at a density of two or more per high-power field (Case 26) (H&E, x400). **C)** Periventricular white matter necrosis, with axonal spheroids (arrows) (Case 3) (H&E, x400). **D)** Acute neuronal necrosis (apoptosis), with hypereosinophilia and nuclear fragmentation (arrows) in the hippocampus/subiculum (Case 3) (H&E, x200).

Table 2: Case Demographics and Findings in Sudden Unexpected Infant Death and Sudden Unexpected Death in Childhood

Case	Postnatal Age at Death	Gestational Age at Birth	Gender	USE	Brain Weight	Cause of Death	Manner of Death	Neuropathologic Findings	Neuropathologic Class	Comments
1	45 min	23 wks	Male	No	90 g	Complications of prematurity	Natural	Acute Germinal matrix hemorrhage	C	Maternal cocaine
2	1 d	23 wks	Male	No	79 g	Complications of prematurity	Natural	Normal brain	C	
3	minutes	30 wks	Female	NA	190 g	ACA, Complications of prematurity	Undetermined	Periventricular leukomalacia (focal white matter necrosis), pontosubicular necrosis	B	Maternal phencyclidine
4	6 wks	32 wks	Male	NA	393 g	Complications of prematurity	Natural	Diffuse white matter gliosis, brainstem gliosis	C	Twin
5	2 mos	29 wks	Female	No	427 g	Undetermined	Undetermined	Germinal matrix cyst, diffuse white matter gliosis, brainstem gliosis	C	Prenatal screen positive for Krabbe Disease
6	1 hr	39 wks	Female	No	418 g	Complications of perinatal asphyxia (placental abruption following external cephalic version for breach)	Therapeutic complication	Diffuse white matter gliosis, brainstem gliosis	C	
7	11 d	38 wks	Female	No	340 g	<i>E. coli</i> urosepsis (congenital vesico-ureteral valve)	Natural	Acute meningitis, acute neuronal ischemia	B	
8	3 mos	28 wks	Female	No	496 g	Meningoencephalitis, likely viral	Natural	Meningoencephalitis, likely viral	A	Apnea in neonatal intensive care unit; see Image 2
9	22 d	NA	Male	Yes	502 g	Asphyxia	Accidental	Diffuse white matter gliosis, brainstem gliosis, Germinal matrix cyst, possible microdysgenesis	C	
10	3 mos	29 wks	Female	Yes	450 g	Bronchiolitis	Natural	Diffuse white matter gliosis, brainstem gliosis	C	Born with patent ductus arteriosus
11	20 d	Term	Male	No	562 g	Undetermined	Natural	Diffuse hypoxic-ischemic encephalopathy, brainstem tegmental necrosis suspicious for Leigh Disease, spinal epidural hemorrhage	A	Aspiration during feed, cardiac arrest, on ventilator seven days; magnetic resonance imaging delayed myelin; retinal hemorrhage
12	59 d	33 wks	Male	No	490 g	Meningoencephalitis, probably viral	Natural	Meningoencephalitis, diffuse white matter gliosis, brainstem gliosis	A	
13	1 mo	Term	Female	Yes	448 g	Undetermined (cosleeping)	Undetermined	Diffuse white matter gliosis, brainstem gliosis	C	
14	1 mo	Term	Female	Yes	485 g	Asphyxia	Accidental	Diffuse white matter gliosis, possible microdysgenesis	C	
15	3 mos	31 wks	Female	Yes	556 g	Undetermined	Natural	Diffuse white matter gliosis, brainstem gliosis	C	

Table 2:Continued

Case	Postnatal Age at Death	Gestational Age at Birth	Gender	USE	Brain Weight	Cause of Death	Manner of Death	Neuropathologic Findings	Neuropathologic Class	Comments
16	37 d	Term	Female	Yes	520 g	Undetermined	Undetermined	Diffuse white matter gliosis, brainstem gliosis	C	Bruising face, body; no brain or retinal hemorrhage
17	20 d	Term	Female	Yes	355 g	Undetermined (cosleeping)	Undetermined	Diffuse white matter gliosis, brainstem gliosis, possible microdysgenesis	C	
18	38 d	39 wks	Male	Yes	531 g	Staphylococcal bronchopneumonia complicating respiratory syncytial virus infection	Natural	Diffuse white matter gliosis, brainstem gliosis, sinus thrombosis, possible microdysgenesis	B	
19	2 mos	NA	Male	Yes	NA	Undetermined (cosleeping)	Undetermined	Diffuse white matter gliosis, brainstem gliosis, possible microdysgenesis	C	
20	2 mos	Term	Female	Yes	577 g	Undetermined (cosleeping)	Undetermined	Diffuse white matter gliosis, brainstem gliosis, possible microdysgenesis	C	
21	4 mos	30 wks	Male	Yes	570 g	Undetermined (cosleeping)	Undetermined	Diffuse white matter gliosis, brainstem gliosis, possible microdysgenesis	C	
22	53 d	Term	Female	NA	460 g	Battered child syndrome	Homicide	Traumatic brain injury, diffuse white matter gliosis, brainstem gliosis, old spinal epidural hemorrhage	A	
23	2 mos	38 wks	Male	Yes	568 g	Undetermined	Undetermined	Diffuse white matter gliosis, brainstem gliosis	C	
24	6 mos	23 wks	Male	No	540 g	Complications of prematurity	Natural	Diffuse white matter gliosis, acute neuronal ischemia	C	
25	3 mos	NA	Male	Yes	680 g	Undetermined	Undetermined	Diffuse white matter gliosis	C	Sibling SIDS
26	3 mos	40 wks	Female	Yes	710 g	Asphyxia	Accidental	Diffuse white matter gliosis, brainstem gliosis, subarachnoid hemorrhage old and recent, possible microdysgenesis	C	
27	3 mos	40 wks	Female	Yes	650 g	Undetermined (cosleeping)	Undetermined	Normal brain, spinal epidural hemorrhage	C	
28	3 mos	40 wks	Female	Yes	610 g	Asphyxia	Accidental	Diffuse white matter gliosis, possible microdysgenesis, spinal epidural hemorrhage	C	
29	4 mos	37 wks	Male	No	673 g	Undetermined	Undetermined	Diffuse white matter gliosis, Germinal matrix cyst, acute neuronal ischemia, possible microdysgenesis, old spinal epidural hemorrhage	C	
30	4 mos	37 wks	Male	NA	662 g	Undetermined	Undetermined	Diffuse white matter gliosis and mineralization, possible microdysgenesis	C	Recent vaccination, patent foramen ovale

Table 2:Continued

Case	Postnatal Age at Death	Gestational Age at Birth	Gender	USE	Brain Weight	Cause of Death	Manner of Death	Neuropathologic Findings	Neuropathologic Class	Comments
31	4 mos	38 wks	Male	NA	858 g	Undetermined	Undetermined	Diffuse white matter gliosis, brainstem gliosis, spinal epidural hemorrhage	C	
32	4 mos	38 wks	Female	Yes	711 g	Undetermined (cosleeping)	Undetermined	Possible microdysgenesis	C	
33	5 mos	34 wks	Male	NA	760 g	Interstitial pneumonia, probably viral	Natural	Diffuse white matter gliosis	C	
34	4 mos	39 wks	Male	No	705 g	Respiratory syncytial virus pneumonia	Natural	Diffuse white matter gliosis, old and recent subarachnoid hemorrhage and subdural hemorrhage, slight, possible microdysgenesis	C	
35	5 mos	Term	Female	NA	679 g	Abusive head trauma	Homicide	Craniospinal trauma, ocular hemorrhage	A	
36	5 mos	38 wks	Female	Yes	850 g	Asphyxia	Accidental	Normal brain	C	Acute life-threatening events
37	7 mos	37 wks	Female	NA	192 g	Undetermined (cosleeping), bronchopneumonia, complications of perinatal asphyxia (uterine rupture)	Undetermined	Ulegyria, laminar necrosis	B	
38	7 mos	NA	Male	NA	939 g	Blunt force injury	Accidental	Traumatic head injury (skull fracture, subdural hemorrhage, subarachnoid hemorrhage)	A	Motor vehicle accident
39	8 mos	38 wks	Female	No	435 g	Bronchiolitis, complications of genetic syndrome (Cri du Chat)	Natural	Micrencephaly, diffuse white matter gliosis and hypomyelin, brainstem gliosis, acute neuronal ischemia, possible microdysgenesis	B	Cri du Chat, laryngomalacia, healing fracture tibia
40	9 mos	39 wks	Male	Yes	930 g	Acute hydrocephalus (Dandy-Walker malformation)	Natural	Acute hydrocephalus (Dandy-Walker malformation)	A	
41	9 mos	41 wks	Male	Yes	935 g	Undetermined (cosleeping)	Undetermined	Acute neuronal ischemia	C	
42	10 mos	Term	Female	No	1020 g	Infectious gastroenteritis	Natural	Germinal matrix cyst, possible microdysgenesis	C	
43	10 mos	Term	Male	NA	398 g	Complications of perinatal anoxia (peripartum uterine rupture)	Natural	Multicystic encephalopathy	B	Seizures
44	10 mos	NA	Female	NA	946 g	Drowning	Homicide	White matter mineralization, brainstem gliosis, acute neuronal ischemia, spinal epidural hemorrhage	C	Drowning

Table 2:Continued

Case	Postnatal Age at Death	Gestational Age at Birth	Gender	USE	Brain Weight	Cause of Death	Manner of Death	Neuropathologic Findings	Neuropathologic Class	Comments
45	13 mos	Term	Male	NA	1100 g	Anoxic complications following drowning in tub	Undetermined	Hypoxic-ischemic myelopathy	B	Drowning; resuscitation and ventilator-dependence, death after seven days
46	13 mos	Term	Male	NA	985 g	Acute asthma with aspiration of gastric contents	Accident	Diffuse white matter gliosis, brainstem gliosis, spinal epidural hemorrhage, slight, possible microdysgenesis	C	Became unresponsive during feeding
47	15 mos	NA	Male	NA	1100 g	Smoke inhalation	Accidental	Acute spinal epidural hemorrhage	C	House fire
48	17 mos	NA	Female	Yes	1173 g	Undetermined	Natural	Hippocampal dysgenesis	B*	Febrile seizure at 14 mos status epilepticus, hospitalized; 2-year old cousin with febrile seizure; two variants of uncertain significance on cardiac genetics; see Image 3
49	18 mos	39 wks	Female	NA	1008 g	Sepsis	Natural	Possible hippocampal dysgenesis, recent subdural hemorrhage, spinal epidural hemorrhage	B*	Cultures lung, <i>Staphylococcus</i> ; nasopharynx, adeno- and rhinovirus
50	24 mos	32 wks	Male	No	1110 g	Undetermined	Undetermined	Hippocampal dysgenesis, brainstem and cerebellar white matter gliosis	B*	Febrile seizure in past, recent fever when put down; see Image 3
51	36 mos	NA	Male	NA	1100 g	Abusive head and neck trauma	Homicide	Hyperextension injury, cervical spinal cord	A	
52	42 mos	NA	Male	NA	1260 g	Multiple blunt impacts, anoxia	Undetermined	Craniospinal trauma, ocular hemorrhage, acute neuronal ischemia	A	
53	43 mos	NA	Male	NA	1130 g	Undetermined	Undetermined	Organizing ischemia, spinal cord; brain anoxia, spinal epidural hemorrhage	B	Respiratory arrest, inflicted vs. accidental trauma

NA - Not available or not applicable

USE - Unsafe sleeping environment or position

ACA - Acute chorioamnionitis

SIDS - Sudden infant death syndrome

* See text for explanation

Clinicopathologic Characteristics of Study Group

During the six-month study interval, we received 53 cases for evaluation. Of these, 44 were infants (age range, minutes to ten months; median, two months) and nine were toddlers (age range, 13 months to 3.5 years; median, 18 months). Twenty-six were girls and 27 were boys. Thirteen were born prematurely between 23 and 36 completed gestational weeks. The gestational age in weeks was not known in all cases; in many it was only known to be “term”, and when no specific mention in the investigator’s or medical examiner’s notes was made, it was assumed to be term.

One infant had a history of acute life-threatening events (ALTEs) prior to demise. One had episodes of apnea in the nursery. Specific neurologic conditions noted in infancy prior to the time of autopsy included Dandy-Walker malformation (n=1; status post ventriculoperitoneal shunt, with recent removal), Cri du Chat syndrome (n=1), and multicystic encephalopathy/ulegyria (n=2; related to catastrophic peripartum anoxia at term).

Two toddlers had a personal history of febrile seizures. One of these (Case 48) had required hospitalization for status epilepticus three months prior to death, and had a 2-year-old cousin with febrile seizures; at autopsy, two variants of uncertain significance (TRPM4 [NM_017636.3:c.2502C>G g.19:49699988C>G] and ANK2 [NM_001148.4:c.5786C>T g.4:114275560C>T]) were noted on our in-house 95-gene cardiac genetic analysis panel. The other (Case 50) had one febrile seizure in the past; the night he was put to bed, he had been noted by the caretaker to have a fever. A third case with hippocampal histology suggestive of dysgenesis (Case 49) was not known to have any prior febrile seizures, but did have autopsy microbiologic cultures positive for *S. aureus*, adenovirus, and rhinovirus.

Of the 12 infants known to be born prematurely, four died of related complications (two within a short time of birth at 23 gestational weeks and two after multiple complications including respiratory and gastrointestinal illnesses). One died within minutes of birth

at 30 gestational weeks in the setting of severe, acute chorioamnionitis. Ten infants (both term and preterm) died of infection (pneumonia, sepsis, gastroenteritis, or meningitis; bacterial or viral).

With regard to unsuspected substrates for sudden death, one infant had a cardiac defect found at autopsy and another had brain changes highly suggestive of Leigh disease, including neuroimaging findings of delayed myelination, although the diagnosis could not be confirmed (specific mitochondrial abnormality testing was not available).

Five infants died of accidental asphyxia related to wedging, overlaying, or occlusive bedding in bed sharing or other positional compromise.

Nine others were in unsafe sleeping environments (e.g., bed sharing and excessive soft bedding; denoted as USE in **Table 2**), but did not have clear evidence of wedging or overlaying; these were ruled “undetermined (cosleeping)” as COD, and of undetermined manner. Six infants were undetermined in both COD and MOD, since autopsy, scene investigation, and complete autopsy with ancillary studies did not reveal specific findings (note that these could be called sudden unexpected infant death, in some jurisdictions, or sudden infant death syndrome, per historical criteria).

Abusive head and/or spinal trauma was documented, along with general autopsy findings and consistent perimortem circumstances, in four cases (two infants and two toddlers). Two children died by drowning, and one in a house fire. One infant died following accidental blunt force trauma (in stroller hit by motor vehicle).

Findings With Regard to Diagnostic Class A, B, or C

Of the 44 infant cases, seven (15.9%) had Class A neuropathologic findings critical to the cause and manner of death. These diagnoses included meningoencephalitis (2/44) (**Image 2**); craniospinal trauma (3/44); brainstem necrosis suggesting Leigh Disease (1/44); and acute hydrocephalus in Dandy-Walker malformation (1/44) (**Table 2**).

Among the toddlers, two of nine (22.2%) had Class A neuropathologic findings, comprising inflicted cranio-spinal trauma (**Table 2**).

Among all infants and toddlers, Class B factors were identified in 11/53 (20.8%), and included recent hypoxic-ischemic lesions (2/11, 18.2%); meningitis or dural venous sinus thrombosis in systemic sepsis (2/11, 18.2%); multicystic encephalopathy/ulegyria following peripartum asphyxia (2/11, 18.2%); hypoxic-ischemic myelopathy, one in (near-)drowning after seven days on mechanical ventilation, and one in undetermined circumstances (2/11, 18.2%); and microcephaly and delayed myelination (Cri-du-Chat Syndrome) (1/11, 9.09%). Class B also included three toddlers (3/11, 27.2%) with features of hippocampal dysgenesis, two in the setting of febrile seizures denoted as B* in **Table 2** (**Table 2** and **Image 3**).

Noncontributory (Class C) features, such as white matter and brainstem gliosis, were identified in 30/53 (56.6%); three of 53 cases (5.7%) had normal brains (**Table 2**). Spinal epidural hemorrhage was noted in ten subjects and, unless accompanied by other definitive evidence of spinal trauma, was considered a Class C finding.

Diagnostic Yield of Histologic Sections

Of the 53 study cases, the recommended neuroanatomic sites were sampled in at least 77.4% (amygdala), up to 98.1% (cerebellum). Those that had more limited sampling (n=6; number of sections submitted, 5-11) tended to be very premature (i.e., have small brain specimens), autolyzed, or otherwise suboptimal. Even among those cases with limited sampling, Class A and B diagnoses could be made (Cases 12, 38; Case 7, respectively).

The most valuable sections in terms of providing information considered Class A or Class B with regard to the determination of the COD and/or MOD were the hippocampi (45.0-50.0% of cases), basal ganglia including internal capsule (40.0%), midbrain and pons (45.0%), medulla (60.0%), cerebral cortical sites (up to 35.0%), and dura (generally when hemorrhages or neomembranes were seen macroscopically, 35.0%) (**Table 1**). Sections of insula and superior temporal gyrus, amygdala, hypothalamus, and cerebellar vermis and hemisphere were of lower yield, offering relevant findings in only 0-20% of cases on routine sectioning per protocol.

Image 2: Meningoencephalitis, likely viral. **A)** Cortical surface and leptomeninges, with reactive changes, including lymphocytic infiltrates and macrophages, as well as pial glial activation (arrows) (Case 8) (H&E, x400); **B)** Parenchymal perivascular lymphocytic infiltrates and nearby microglial nodule (arrow) (Case 8) (H&E, x400).

Eyes and/or optic nerves and cervical cord/vertebral blocks with nerve roots were obtained by the referring medical examiner in ten and three cases, respectively. Of note, these specimens were generally obtained in cases with a high index of suspicion (rather than being routinely taken as a default); thus, they were not included in the analysis of diagnostic yield.

DISCUSSION

The NYC OCME is a busy center performing over 5000 full autopsies per year. As a government (i.e., taxpayer-supported) entity, we are under continual pressure to justify our resource allocation, while also being expected to care for families and protect the public health. Thus, we depend upon evidence-based

analyses of our practices to determine the optimal diagnostic yield for our efforts regarding certain types of deaths among the population we serve. It was our goal, in this study, to survey an unselected series of infant and toddler cases received at our office across a six-month interval in order to determine an overall best practice with regard to the extent of the neuropathologic examination needed to influence the final formulation of the cause and manner of death.

We applied a comprehensive, uniform sectioning protocol to all infant and toddler central nervous system specimens, and tallied the instances in which the neuropathology examination was critical (Class A); contributory (Class B); or noncontributory (Class C) to the medical examiner's formulation of the COD and

Image 3 A: Hippocampal dysgenesis. Coronal section of cerebral hemispheres at level of lateral geniculate nuclei, demonstrating asymmetry of the hippocampal formations. Note the more vertical orientation of the left hippocampus (left side of image), as compared to the right (Case 48).

Image 3 B: Hippocampal dysgenesis. The dentate gyrus shows marked irregularity in thickness with focal duplication (bilamination) (arrows) (Case 50) (H&E, x200).

MOD. Our findings indicate that a substantial proportion of cases—about 17% across infant and toddler deaths—had neuropathologic abnormalities having crucial impact on the final COD and MOD. An additional 21% had neuropathologic findings playing a somewhat influential role in the final formulation of cause and manner. These results stand in contrast to the study of Pryce et al., in which only 6% of SUID cases had gross and/or microscopic neuropathologic findings of importance to the final diagnosis (15). That study differed, however, in important ways from ours. Although it was a very large study, comprising 724 SUID cases having both macroscopic and microscopic findings accrued over 14 years, the neuropathologic examinations were conducted entirely by pediatric pathologists in 38% and by pediatric pathologists macroscopically and by neuropathologists microscopically in 36%, with only 26% being completely worked up by neuropathologists. Moreover, the study was retrospective and therefore lacked the uniformity of approach and consistent expertise leveraged in our study. Of note, the range of neuropathologic findings in that study was similar to ours, including meningitis and hydrocephalus. Their concluding points were that a *“macroscopically abnormal brain and the presence of a clinical history of possible neurological disease or of inflicted injury are significantly more likely to be associated with significant histological brain abnormalities”* (15). While we essentially are in agreement, we demonstrate a higher percentage of cases in which the neuropathologic examination was influential. We further offer a refinement of the default section protocol, based on the impact of microscopic evaluation of specified regions on the final determination of cause and manner of death.

In line with many prior reports in SUID (also referred to as SIDS in the historical literature), we also noted relatively nonspecific neuropathologic changes, including gliosis of the deep cerebral white matter and brainstem, and acute neuronal ischemia, in over 50% of our cohort. Such subtle white matter and brainstem gliosis has been interpreted as a marker of hypoxia-ischemia, perhaps occurring in the setting of observed or occult ALTEs (16). Of note, one infant in our cohort had a clinical history of ALTEs, and another

had apnea in the intensive care nursery; however, neither had appreciable gliosis according to the criteria set forth in the Methods.

Spinal epidural hemorrhage was fairly common (10/53 cases), and showed no pattern of association with the dominant pathologic process related to COD and MOD. This finding is in alignment with the review of Ali and Fowler (17).

Hippocampal dysgenesis, as defined by Kinney and colleagues, first in toddlers with febrile seizures (12), and more recently in infants (8, 13), has yet to be replicated as a diagnostic entity in other large series. Nevertheless, we felt compelled to make the neuropathologic diagnosis in two of our toddlers with clinical histories of febrile seizures, on the basis of macroscopic asymmetry of the hippocampal formations as well as microscopic features, chiefly dispersal and bilamination of the dentate gyrus. We also had a third toddler with pathologic features suggestive of hippocampal dysgenesis, but without a known personal or family history of febrile seizures. During the course of this study, we came to consider these cases as “Class B*” (Table 2) to highlight the recent evidence that hippocampal dysgenesis may represent a substrate for sudden death across the spectrum of childhood (8), while recognizing that medical examiners may still be relatively unfamiliar with the potential implication of this finding with respect to their final COD and MOD determinations. We speculate that, with experience, these cases may come to be assigned to Class A (i.e., the hippocampal abnormalities may be recognized to cause, or at least indicate a marker of significant predisposition to, sudden death). Additional studies of such cases will be necessary to confirm or refute this speculation.

Less clear-cut (i.e., more subtle) variability in the microscopic anatomy of the hippocampus (e.g., thickness of the dentate gyrus with dispersal and/or attenuation, or looping of dentate cells around a vessel) was noted in a subset of our cohort, and is undergoing separate detailed analysis with regard to potential clinical significance.

Of note, those cases assigned by us as Class C, though not directly contributory to COD or MOD, nonetheless have value in excluding underlying substrates in SUID and SUDC. We emphasize that following the suggested sectioning protocol lent a degree of confidence that no significant finding was missed in what was undertaken to be a complete autopsy.

With respect to the value of specific neuroanatomic sampling in the determination of COD and MOD in our study population, we found that the following sections tended to have the greatest diagnostic yield, assisting in detection of inflammation (infection), hypoxia-ischemia, or malformation: hippocampus (both sides if asymmetric, or in the setting of concern for

febrile seizures); cerebral cortex and leptomeninges; basal ganglia; and brainstem, especially medulla. Sections of dura were useful for dating of associated hemorrhage, when present. We emphasize that all other neuroanatomic locations mentioned in the protocol in **Table 1** should be set aside in stock jar(s), in the event that later evaluation becomes necessary (see **Image 4**). We also highly recommend that the brain be fixed for at least some days (or up to three weeks) to allow the best sections to be prepared for microscopic analysis. With regard to sampling of eyes and cervical vertebral blocks, we did not include diagnostic yield analysis of them in this study, and at this time continue to recommend the use of discretion by individual forensic pathologists in choosing to obtain them.

Image 4: Blocking diagram for histologic sectioning in sudden unexpected infant death and sudden unexplained death in childhood cases, numbered according to the section number in **Table 1**. Solid-line boxes indicate those sections having the highest diagnostic yield, as tallied in **Table 1**. Dashed-line boxes are those taken in addition, per the sectioning protocol of Robert's Program, and which should ideally be taken for stock jar, should further in-depth analysis be needed. (Not shown: spinal cord, for which cervical, thoracic, and lumbosacral levels may be submitted in one section; see text and references).

Limitations and Strengths of Study

We acknowledge certain limitations in our study, particularly the somewhat subjective nature of determining diagnostic impact class in a given case. We tried to set forth our criteria *a priori*, and required consensus among all authors after reviewing all circumstances of each case. However, other practitioners may bring a different set of experiences and judgment to bear on this determination. Our cohort also consists of an admittedly relatively small number of cases.

Despite these potential weaknesses, we highlight some important strengths. First, we point out the value of the uniformity of our approach to all cases in an unselected series, using a sectioning protocol recommended by a recognized expert in the field of sudden unexpected death in pediatrics, Dr. Hannah C. Kinney, who trained and has been a long-time collaborator of one of us (RDF). This approach likely minimized variability in selection of anatomic blocks, as well as in interpretation of neuropathologic changes, which we defined at the outset. All blocks were processed in a single, experienced laboratory at the NYC OCME. With this approach, it was our hope to provide guidance for the larger forensic pathology community with regard to the optimal approach to examination of the nervous system in these challenging cases.

CONCLUSION

It was our goal to develop an evidence-based rationale for a standard neuropathologic workup in cases of sudden unexpected infant and childhood death. To that end, we analyzed the proportion of such cases in which the nervous system was of key importance in determining the COD and/or MOD. We found that as many as one in five cases had significant abnormalities of the nervous system impacting on the final understanding of the cause and manner of death, reinforcing the need for careful and thorough evaluation of the brain, spinal cord, and their coverings. Moreover, we identified a practical subset of sections for microscopy for optimal yield of such key findings. Finally, we add limited evidence that, at least in toddlers, hippocampal dysgenesis in the setting of febrile seizures may be

worthy of recognition as an underlying mechanism of death *sui generis*.

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