JOURNAL OF NEUROTRAUMA 29:672–677 (March 1, 2012)

DOI: 10.1089/neu.2011.1861

Common Data Elements for Pediatric Traumatic Brain Injury: Recommendations from the Biospecimens and Biomarkers Workgroup

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Abstract

Biospecimens represent a critically important resource in pediatric brain injury research. Data from these specimens can be used to identify and classify injury, understand the molecular mechanisms underlying different types of brain injury, and ultimately identify therapeutic targets to tailor treatments for individual patient needs. To realize the full potential of biospecimens in pediatric traumatic brain injury (TBI), standardization and adoption of best practice guidelines are needed to ensure the quality and consistency of specimens. Multiple groups, including the National Cancer Institute (NCI), the International Society for Biological and Environmental Repositories (ISBER), and the Organisation for Economic Co-operation and Development (OECD), have previously published best practice guidelines for biospecimen resources. Recommendations have also been provided by the Biospecimens and Biomarkers Workgroup of the interagency TBI Common Data Elements (CDE) initiative. The recommendations from all of these sources, however, focus exclusively on adult biospecimen collection. There are no published pediatric-specific biospecimen collection guidelines. An additional workgroup was formed to specifically address this gap. The aim of the Pediatric TBI CDE Biospecimens and Biomarkers Workgroup was to provide recommendations for best practice guidelines to standardize the quality and accessibility of biospecimens for pediatric brain injury research in general, and for pediatric TBI research in particular. Consensus recommendations were developed by review of previously published adult-specific recommendations, including the recommendations of the original TBI Common Data Elements Biospecimens and Biomarkers Workgroup, and by participation in the interagency workshop "Common Data Elements for TBI Research: Pediatric Considerations," held in Houston, Texas in March of 2010. These recommendations represent expert opinion on this subject. The authors of this article were members of the Biospecimens Workgroup. We hope that with adoption of these best practices, future investigators will be able to obtain biospecimens in a consistent way that meets the needs of pediatric patients, and helps to accelerate acquisition of pediatric-specific biomarker data.

Key words: best practices; biomarkers; common data elements; pediatrics; standardization; traumatic brain injury

Introduction

Biospecimens represent a critically important and underutilized resource in pediatric traumatic brain injury (TBI). Data from these specimens can be used to identify and classify injury, understand the molecular mechanisms underlying different types of TBI, and ultimately identify therapeutic targets to tailor treatments to individual patient needs. To realize the full potential of biospecimens in pediatric TBI, standardization and adoption of best practice guidelines are needed to ensure the quality and consistency of specimens. Multiple groups, including the National Cancer Institute (NCI), the International Society for Biological and Environmental Repositories (ISBER), and the Organisation for Economic Co-operation and Development (OECD) have previously published best practice guidelines for biospecimen resources (Eiseman et al. 2003; International Society for Biological and Environmental Repositories, 2005; National Institute of Health and Department of Health and Human Services, 2007). Recommendations have also been provided by the Biospecimens and Biomarkers Workgroup of the interagency TBI Common Data Elements (CDE) initiative

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(Manley et al., 2010). The recommendations from all of these sources focus exclusively on adult biospecimen collection. There are no published pediatric-specific biospecimen collection guidelines. As with so many other aspects of TBI research, children with TBI are not just "little adults" (Giza et al,. 2007), and biospecimen collection in children is more complex than simply decreasing the volume of specimens that are collected in adults. An additional workgroup was formed to specifically address this gap: the Pediatric TBI CDE Biospecimens and Biomarkers Workgroup. As with the original CDE workgroup, physicians with specific expertise in biomarkers research were recruited to participate in the Pediatric CDE Workgroup. We also recruited an expert in issues related to human subjects concerns/research ethics as it relates to pediatric biospecimens issues.

Further information regarding the background of the TBI CDE initiative (Thurmond et al., 2010), and the methods used by all workgroups to arrive at CDE recommendations is detailed by Miller and colleagues (Miller et al., 2011).

The purpose of this article is to complement the recommendations of the original TBI CDE Biospecimens Workgroup (hereafter referred to as the "original CDE"; Manley et al., 2010), by addressing pediatric-specific and pediatric TBI-specific issues in biospecimen collection. In addition, we anticipate that researchers in pediatric brain injury, including epilepsy, stroke, and perhaps neonatology, may also find these guidelines helpful.

Aim

The aim of the workgroup was to provide pediatric-specific recommendations for core, supplemental, and emerging biospecimen and biomarker CDE for TBI research, and to develop best practice guidelines to standardize the quality and accessibility of these specimens in pediatric subjects.

Approach

Previously published statements related to biospecimen and biomarkers collection were reviewed, as were the recommendations from the original CDE (Manley et al., 2010). There were several face-to-face meetings, as well as multiple e-mail interactions between the co-authors. Preliminary recommendations were presented at the TBI Common Data Elements: Pediatric Considerations workshop in Houston, Texas in March 2010, with feedback resulting in subsequent modifications to the recommendations. There was agreement that the focus of the current article would be on the differences between the original CDE related to biospecimens and biomarkers, and the current pediatric recommendations.

Recommendations for CDE for pediatric biospecimens and biomarkers

The group's recommendations are divided into two sections, each having several subsections: (1) biomarker CDE, and (2) biospecimens.

Biomarker CDE

Consistent with the original CDE workgroups and with the other pediatric CDE workgroups, this Pediatric TBI Biospecimens and Biomarkers Workgroup adopted the standard three-category classification system in its selection of CDE (Miller et al., 2011). In the first category, core biomarker CDE are intended to encompass the minimal set of measures to characterize a broad spectrum of subjects in the domain. Supplemental biomarker CDE are intended for greater depth and breadth of exploration, and/or more specialized subpopulations. Emerging biomarker CDE may require further validation, but may fill gaps in currently validated measures, and/or substitute for recommended measures when validation is complete. As with the original CDE, there are no recommendations regarding the specific biomarkers that should be studied.

Core CDE recommendations

Collection of an acute (< 24 h after injury) serum sample for proteomic and metabolomic analyses. We recommend that parental consent be obtained according to local institutional review board (IRB) procedures to answer the specific question under study, as well as to allow the investigators to utilize these samples for exploratory future studies. As discussed below in the section on sample collection, the blood volume for pediatric subjects will often be substantially lower than that utilized in studies of adult subjects.

In contrast to the original CDE, we do not recommend collection of DNA samples for genomic analysis as a core data element. While there are multiple adult studies that support an effect of the apoE-ε4 genotype on outcomes of TBI (Alexander et al., 2007; Crawford et al., 2002; Friedman et al., 1999; Ost et al., 2008), the current literature for these effects in children is quite limited and not sufficiently compelling to allow us to recommend this collection as a core data element (Moran et al., 2009). From an ethical standpoint, there is significant controversy regarding the collection of genetically-identifiable material from children. The long-term ramifications of DNA collection for a child and his or her family are complex and will likely change over the child's lifetime. Although the 2009 Genetic Information Nondiscrimination Act (GINA) makes it illegal for certain groups (i.e., health insurance companies, group health plans, and some employers) to discriminate based on genetic information, anticipating reasonably foreseen risks as required by Health and Human Services regulations is difficult when children are involved (Office for Human Research Protections and Department of Health and Human Services, 2009). Thus, for both scientific and ethical reasons, we cannot recommend collection of DNA for genomic analysis as a core element at this time. We would, however, encourage DNA collection if it is part of a hypothesis-driven pediatric study, and recommend that the consent form address the specific use of DNA for that particular study, and contain a statement describing how the confidentiality of subject records will be maintained.

Supplemental CDE recommendations

Collection of serial serum and CSF samples for proteomic analysis. We agree with the original CDE recommendation to collect serial serum and cerebrospinal fluid (CSF) samples (Manley et al., 2010). As discussed below in the section on sample collection, pediatric-specific issues related to blood volume are even more important in the case of serial sampling.

Extended CDE recommendations

Collection of cerebral microdialysis samples. We agree with the original CDE recommendation for cerebral microdialysis specimens as an extended data element. Few

pediatric data exist at this time, and any study using cerebral microdialysis samples would need to be hypothesis-driven. In contrast to the original CDE recommendations, we would not recommend collection of peripheral blood mononuclear cells (PBMCs) for gene and protein expression studies at this time. There are currently no pediatric data to support collection of PBMCs after TBI, and the volume required is often significantly greater than would be allowable under the IRB restrictions discussed below. Advances in the use of PBMCs in children will likely begin with the use of umbilical venous blood, a situation in which sample volume is not a concern.

Developmental considerations

Measurement of biomarkers in the pediatric population requires temporal and developmental considerations. Since children's brains are undergoing continued development throughout adolescence, the normal concentrations of neurological markers may change over time as part of normal physiological processes. One of the most well-recognized examples of an age-dependent marker is S100B. Serum concentrations of S100B, a marker of astroglial cell death, are inversely proportional to age, with the youngest children having the highest concentrations (Gazzolo et al., 2003b; Portela et al., 2002). It has been hypothesized that age-related changes in the blood–brain barrier, and/or age-dependent release of S100B from adipocytes and chondrocytes, may be the cause of the age-dependence seen with this marker (Donato, 2007; Goncalves et al., 2010; Holtkamp et al., 2008; Netto et al., 2006).

In addition to the need to consider subject age at the time of study enrollment, it is important to consider that in studies in which blood samples are collected over a period of months or years, changes in biomarker concentrations may occur due to developmental, rather than pathological, changes. Because of these issues, it is important that researchers consider age as a potential confounder prior to the start of a given study, and consider using age-matched controls.

Biospecimen collection, processing, documentation, and storage

Biospecimen collection

Blood sample volume. The original CDE workgroup recommended that 5–10 mL of blood be collected for each sample. For pediatric subjects, we recommend that the volume collected be consistent with a pre-defined standard that meets at least the minimal IRB requirements. This is particularly important for serial sampling and for infants and young children. A standard table of allowable volumes is publically available, and has been accepted by several IRBs, including the University of Pittsburgh, Children's Hospital in Los Angeles, Baylor College of Medicine in Dallas, and Cincinnati Children's Hospital (Table 1). It is important to recognize that the blood volumes in the table include blood collected both for research and clinical purposes. In a 4-kg child, for example, the total allowable blood volume for research and clinical care in a single blood draw is 8 mL, and the total allowable blood volume for research and clinical care in a 30-day period is 16 mL. Because of these limitations on blood volume, it can be helpful to obtain IRB approval to use serum that is left over from clinical samples for research purposes. This blood would otherwise be discarded, and can be used for biomarker analysis as long as the processing and storage is appropriate as described below.

Risks of phlebotomy. In a child with severe TBI, it is likely that arterial or central venous access will be available and phlebotomy will not be necessary. However, in children with mild to moderate TBI and in control subjects, phlebotomy may be necessary. While phlebotomy is considered a minimal-risk procedure from an IRB perspective, the pain and discomfort for a child undergoing phlebotomy must be taken into account and stated in any consent/assent form. We would recommend that as part of the protocol/consent, there is a limit to the number of phlebotomy attempts that can be performed (i.e., preferably 1 or 2) in a child who cannot assent to the procedure (generally children <7 years of age). In cases in which time permits, we would recommend that consideration be given to the use of a topical anesthetic prior to phlebotomy. Some IRBs require the use of a topical anesthetic as a way to minimize the pain associated with blood draws.

Site of sample collection. We agree with the original CDE that documentation of the collection site is important. While adult samples are generally limited to venous and arterial samples, capillary specimens are also a possibility in pre-mobile children. If the heel is pre-warmed and collection is done by an experienced phlebotomist, it is possible to collect 1 mL of blood from a heel stick in a pre-mobile child. However, it should be noted that no published data have directly compared biomarker concentrations in capillary and venous specimens.

Although not discussed as part of the recommendations of the original CDE, urine is another potential biospecimen that may be useful for biomarkers that are renally excreted. Urinary S100B concentrations have been evaluated in one pediatric TBI study, and extensively in neonates with hypoxic-ischemic encephalopathy (Berger and Kochanek, 2006; Gazzolo et al., 2001,2003a).

Hemolysis. Sample hemolysis is not discussed in the original CDE recommendations, but is an important issue in the pediatric patient because of the small needle gauge often used for phlebotomy. In addition, capillary specimens are frequently hemolyzed. Hemolysis can have an important effect on the concentrations of brain biomarkers, mostly notably neuron-specific enolase (NSE). NSE is found in small quantities in red blood cells, and thus sample hemolysis can result in falsely elevated serum NSE concentrations. Previous research has demonstrated that qualitative assessment of the amount of hemolysis is not accurate (Berger and Richichi, 2009). In the case of NSE, it is possible to adjust the NSE concentration to account for the amount of hemolysis by using a quantitative assessment of the amount of hemolysis and an adjustment factor (Berger and Richichi, 2009). This type of adjustment factor has not been derived for other biomarkers.

Cerebrospinal fluid. We agree with the recommendations of the original CDE as they relate to CSF collection. CSF can be obtained from an indwelling catheter in the ventricular space (externalized ventricular drain [EVD]), or via lumbar drain or puncture. CSF specimens obtained via EVD should be collected using established sterile technique. Lumbar drains, infrequently placed in childhood TBI patients, can offer another

Table 1. Maximum Allowable Total Blood Draw Volumes (Clinical and Research)

Body weight (kg)	*Body weight (lb)*	*Total blood volume (mL)*	*Maximum allowable volume (mL) in one blood draw (2.5% of total blood volume)*	*Maximum volume (clinical and research) (mL) in a 30-day period*	*Minimum hemoglobin required at time of blood draw*	*Minimum hemoglobin required at time of blood draw if subject has respiratory or CV compromise*
1	2.2	100	2.5	5	7.0	9.0–10.0
2	4.4	200	5	10	7.0	9.0–10.0
3	6.3	240	6	12	7.0	9.0–10.0
4	8.8	320	8	16	7.0	9.0–10.0
5	11	400	10	20	7.0	9.0–10.0
6	13.2	480	12	24	7.0	9.0–10.0
7	15.4	560	14	28	7.0	9.0–10.0
8	17.6	640	16	32	7.0	9.0–10.0
9	19.8	720	18	36	7.0	9.0–10.0
10	22	800	20	40	7.0	9.0–10.0
11–15	24–33	880–1200	22–30	44–60	7.0	9.0–10.0
16–20	35–44	1280–1600	32–40	64–80	7.0	9.0–10.0
21–25	46–55	1680–2000	42–50	64–100	7.0	9.0–10.0
26–30	57–66	2080–2400	52–60	104–120	7.0	9.0–10.0
31–35	68–77	2480–2800	62–70	124–140	7.0	9.0–10.0
36–40	79–88	2880–3200	72–80	144–160	7.0	9.0–10.0
41–45	90–99	3280–3600	82–90	164–180	7.0	9.0–10.0
46–50	101–110	3680–4000	92–100	184–200	7.0	9.0–10.0
51–55	112–121	4080–4400	102–110	204–220	7.0	9.0–10.0
56–60	123–132	4480–4800	112–120	224–240	7.0	9.0–10.0
61–65	134–143	4880–5200	122–130	244–260	7.0	9.0–10.0
68–70	145–154	5280–5600	132–140	264–280	7.0	9.0–10.0
71–75	156–185	5680–6000	142–150	284–300	7.0	9.0–10.0
76–80	167–176	6080–6400	152–160	304–360	7.0	9.0–10.0
81–85	178–187	6480–6800	162–170	324–340	7.0	9.0–10.0
86–90	189–198	6880–7200	172–180	344–360	7.0	9.0–10.0
91–95	200–209	7280–7600	182–190	364–380	7.0	9.0–10.0
96–100	211–220	7680–8000	192–200	384–400	7.0	9.0–10.0

CV, cardiovascular.

reservoir of potential samples. Lumbar puncture is almost never indicated in children with TBI. However, this technique can be used in control subjects (generally undergoing diagnostic procedures) to obtain CSF for analysis. We would, however, recommend that if researchers are considering collection of CSF via lumbar puncture from healthy children who are not undergoing diagnostic procedures, that this be discussed directly with the local IRB. The decision of individual IRBs might be expected to vary in their risk determination of pediatric studies that include CSF collection, since healthy children cannot undergo greater than minimal-risk procedures in any research study. Investigators should be prepared to provide a rationale that discusses how the risk is justified by the extent of the potential benefit to the involved children, and if the procedure does not have the potential for direct benefit, that this risk represents only a minor increase over minimal risk (45 CFR 46, Subpart D, sections 401-409; U.S. Department of Health and Human Services, 1993).

Biospecimen processing

We agree with the recommendations of the original CDE as it relates to sample processing, with one small change related to sample volume. With multiplex bead technology, the volumes required for biomarker measurement are often <100 μL, and therefore an aliquot of 1–2 mL could result in multiple freeze-thaw cycles before the sample would be exhausted. We therefore recommend aliquots of 250 μL. This recommendation is based on an assessment of an acceptable balance between the need to limit the number of freeze-thaw cycles, and the need to limit the amount of freezer space necessary as more aliquots are made for each subject sample.

Biospecimen documentation and storage

Documentation. One of the key CDE that must be recorded for each sample is the time after injury when the sample is collected. This is particularly important for biomarkers with a short half-life, such as S100B. In order to calculate the time after injury, it is necessary to have both a time of injury and a time of sample collection. In cases of abusive head trauma, an important cause of TBI in infants and young children, the time of injury is rarely known. As a result, we would recommend that in cases of suspected abuse, or in other cases in which the time of injury is not known, the time of injury be set in a consistent fashion in relation to the time of first contact with medical personnel (e.g., a call to emergency medical services [EMS], EMS arrival, or arrival to a hospital in cases in which EMS is not utilized). Estimating the time of injury in this way provides consistency and allows for comparison between centers.

Storage. We agree with the original CDE recommendations related to sample storage with one important difference.

Because the Office for Human Research Protection (OHRP) considers informed consent to be an ongoing process, unless the IRB determines that requirements for obtaining informed consent can be waived, investigators are required to obtain consent from the subject when he or she reaches age 18. This regulation applies to research with biospecimens because this type of study may involve the continued analysis of identifiable specimens (e.g., by linkage code). Thus, biospecimens obtained before the age of 18 must be discarded unless the subject either re-consents for the samples to be kept, or the samples are rendered anonymous, meaning that all links to identifiable data are removed. As a result of this regulation, we recommend that at the time of sample collection, there be a plan in place for tracking subject age so that it is clear when each subject reaches his or her 18th birthday, and for re-consenting, anonymizing, or discarding all specimens at that time.

Future Directions

Adoption of standard practices for sample collection, processing, and storage, and for collection of biomarker-related data elements, will advance pediatric TBI research. We agree with recommendations of the original CDE workgroup, that a pilot study would help to demonstrate the feasibility of these recommendations, and identify which recommendations require clarification. As in the adult biomarker community, successful implementation of the recommendations will require dissemination of the recommendations, engagement of the research community, and solicitation of comments and feedback. The small number of researchers performing pediatric biomarker research will almost certainly make this outreach less difficult than in the adult community. As with the adult-specific recommendations, it is likely that as the field advances, there will need to be periodic reassessment and modification of these recommendations.

Acknowledgments

We gratefully acknowledge the valuable assistance of Haley Stutz for assistance with manuscript preparation.

The views expressed are those of the authors and do not necessarily reflect those of the agencies or institutions with which they are affiliated, including the U.S. Department of Veterans Affairs, the U.S. Department of Education, and the National Institutes of Health. This work is not an official document, guidance, or policy of the U.S. government, nor should any official endorsement be inferred.

This project was jointly supported by the National Institutes of Health–National Institute of Neurological Disorders and Stroke, and the U.S. Department of Education/National Institute on Disability and Rehabilitation Research.

Author Disclosure Statement

No competing financial interests exist.

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