JOINT TRAUMA SYSTEM CLINICAL PRACTICE GUIDELINE

Use of Traumatic Brain Injury Biomarkers after a Potentially Concussive Event

Guidelines for use of Whole Blood (WB) biomarkers in the evaluation of casualties with mild TBI (concussion) in a deployed environment.

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Original publication date: 16 Aug 2021	Publication Date: 14 Apr 2025	Supersedes: 16 Aug 2021

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SUMMARY OF CHANGES

1. Title changed from "Use of Traumatic Brain Injury Plasma Biomarkers after a Potentially Concussive Event (CPG ID:90)" to "Use of Traumatic Brain Injury Biomarkers after a Potentially Concussive Event (CPG ID:90)" to reflect FDA approval of the Abbott whole blood test cartridge in March 2024. The existing plasma cartridge version, applicable to the existing CPG, will not be fielded and guidelines on its use have been removed.
2. The term "plasma" was changed to "whole blood" throughout document.
3. The time period post injury in which the assay is approved to be used was changed from 12 hours to 24 hours throughout document.
4. Background: New assay cartridge added
5. Structure to Support Optimal Use of TBI Biomarker: Use of ADVISOR system added in the case that a Role 3 TBI/Neuro consultant is not available. Reference to Appendix D (and original Appendix D: Biomarker Data Collection Tool) removed.
6. Initial Evaluation: Clarifications of the use of the MACE2 and Red Flags.
7. Moderate Risk: Test TBI Whole Blood Biomarkers Before Evacuation: Removed Appendix D reference, added use of ADVISOR system.
8. Locations with On-Site Head CT: Removed discussion of Appendix D-Biomarker Data Collection Tool.
9. Primary Concussive Blast: Removed discussion of Appendix D
10. Appendix A: Time post injury changed from 12 to 24 hours.
11. Appendix B: Detailed Information on the TBI Whole Blood Biomarker: Changes relevant to the Whole Blood cartridge added, info relevant to Plasma cartridge removed. Study #3 (Whole Blood Pivotal Trial) data added. Intended Use Statement modified to FDA Whole Blood approved IFU.
12. Appendix C: Summary of Research: Updated/info from Study #3 (whole blood pivotal) added.
13. Appendix D: Changed from "Biomarker Data Collection Tool", which was removed (was intended for use during the Clinical Implementation and Operational Assessments at selected sites in Iraq-this was completed).

Use of Traumatic Brain Injury Biomarkers after a Potentially Concussive Event

- *82% of TBI in US Personnel have a final diagnosis of Mild TBI (mTBI)*
- *Head CT usually "normal" in mTBI → 6-8% IC hemorrhage on CT scan/1-2% required neurosurgical intervention*
- *78% of mTBI RTD after evacuation for head CT which was negative (risk to mission/evac personnel)*

POTENTIALLY CONCUSSIVE EVENT

- Vehicle: Blast/Collision/Rollover
- Blast: Within 50 meters or 2 + blast exposures
- Blow to Head: Witnessed loss of consciousness

INITIAL EVALUATION

- ACLS/TCCC Protocols → evac for CT as indicated
- GCS ≤ 12 → Traumatic Brain Injury and Neurosurgery in the Deployed Environment CPG
- GCS 13-15/No Major Injuries → RISK EVALUATION
  - Appendix A algorithm

SCREENING MECHANISMS

- MACE2/ DoDI 6490.11(GCS 13-15)
  - "Red Flags → indication for CT
  - Progressive Return to Activity
- **TBI WB Biomarkers**
- Glial Fibrillary Acidic Protein
- Ubiquitin Carboxyl-terminal Hydrolase L1
  - Released with neuronal damage
  - "Not elevated" has nearly 100% NPV

REQUIREMENTS FOR TBI WB BIOMARKERS

- **ATBI System**
  - iSTAT Alinity System with TBI WB cartridges
  - On-site refrigeration for test cartridges
  - Frozen storage for calibration fluids
- **Expertise**
  - Trauma Medical Director for AOR coordination
  - TBI Biomarker consultant (R3)/ OR
  - ADVISOR teleconsultation system
  - Appendix A algorithm

(if available at your location <24hrs of injury)

TBI Biomarkers Analogous to MACE2 Red Flag: "Structural Brain Injury Detection Device"

- Use only iSTAT Alinity → Elevated → Head CT/ contact Biomarker Consultant
  ↘Not Elevated → MACE 2 /Progressive Return to Activity/ contact Biomarker Consultant
- CT at provider discretion despite negative MACE 2 Red Flag indications → TBI Biomarker test prior to CT
- Location with CT in house → consider drawing TBI Biomarkers (<24hrs) AND CT to gain experience/data

RISK EVALUATION:CLINICAL ALGORITHM FOR TBI BLOOD BIOMARKERS USE (APPENDIX A)

High Risk → No Biomarker Urgent Head CT

- Deteriorating GCS (≥ 2)
- Combativeness/agitation
- Vomiting (≥ 2)
- Seizure
- Focal Neuro Deficits
- Penetrating Brain Injury
- Basilar Skull Fracture

Moderate Risk → TBI Biomarker /Consultant (<24hr of injury)

- Double vision
- Restlessness
- Vomiting (<2)
- Subjective neuro deficits only
- Worsening HAs
- Age > 60
- Antiplatelet meds/EtOH/Drugs
- Amnesia >30min after injury

Low Risk → No Risk Factors

- Care as described in MACE2
- Pt deteriorates after 24hrs
  - Re-contact TBI Biomarker consultant

METRICS

- ✓ MACE 2 Exams documented on all SMs diagnosed with mTBI
- ✓ Results of TBI Biomarker documented on patient's record

This information is pulled from the evidence-based Joint Trauma System (JTS) Use of Traumatic Brain Injury Biomarkers after a Potentially Concussive Event Clinical Practice Guideline (CPG). JTS CPGs can be found at the JTS CPG website or the JTS Deployed Medicine site.

PURPOSE

These guidelines are intended to provide a basic management approach after a potentially concussive event using the specific whole blood (WB) biomarkers for traumatic brain injury (TBI) on the iSTAT Alinity. This guidance does not apply to other brain injury devices or TBI biomarkers. This CPG is intended to complement and build upon the existing DoD Instruction (DoDI) 6490.11 DoD Policy Guidance for Management of Mild Traumatic Brain Injury/Concussion in the Deployed Setting.

BACKGROUND

- Between 2000 and 2023 TBI affected over 499,852 service members worldwide, with 82.2% of those injuries being classified as mild (mTBI), also known as concussion.[1] Common sequelae after mTBI include: headache, visual impairment, Post-Traumatic Stress Disorder (PTSD), depression and cognitive disorders; individuals with a mTBI are at increased risk of posttraumatic stress.[2-4] Secondary to these effects, only 70% of individuals diagnosed with mTBI return to full duty.[5]
- Individuals with mTBI typically have a normal (or negative) head CT. The purpose of head CT in an individual with suspected mTBI is to rule out a more severe injury requiring a higher level of care. For example, intracranial hemorrhage requires inpatient observation and, if severe, neurosurgical intervention. In civilian settings, approximately 6-8% of individuals with suspected mTBI at initial evaluation have evidence of intracranial hemorrhage and 1-2% require neurosurgical intervention[.6,7]
- CT scanners are typically available in theater at Role 3 facilities and select enhanced Role 2 facilities. The decision to transport a casualty from Role 1 /2 to Role 3 for a head CT can have significant implications for the safety of the flight crew and mission operations. Medical decision making should include an assessment of operational risk.
- An evaluation of the DoD Trauma Registry (DoDTR) suggests 68% of casualties identified as having mTBI were evacuated to the Role 3 for a head CT and 41% to Role 4, with approximately 78% of those Service Members being returned to duty.[8] However, it is important to recognize the DoDTR only contains a subset of more severely injured Service Members with a mTBI in theater. Nonetheless, the data suggests that many evacuations for head CT may be avoided.
- The Military Acute Concussion Evaluation 2 (MACE2) provides guidance in the initial evaluation and management of individuals with Glasgow Coma Scale (GCS) score 13-15 in the deployed setting. It includes an assessment of "red flags" to determine the need for head CT and evacuation. The biomarker assay is not intended to replace the MACE2. In addition, the New Orleans Criteria and the Canadian Head CT rule also aid in identifying individuals most likely to benefit from head CT. None of these rules have been validated in the deployed setting.[9,10]
- Glial Fibrillary Acidic Protein (GFAP) and Ubiquitin Carboxyl-terminal Hydrolase L1 (UCH-L1) are contained within cells in the central nervous system and released upon neuronal damage. Higher levels indicate worsening neuronal injury.
- In March 2024, the U.S. Food and Drug Administration (FDA) approved a whole blood biomarker test for TBI, the i-STAT TBI WB Cartridge with the i-STAT Alinity System. This semi-quantitative assay detects levels of GFAP and UCH-L1 in venous whole blood. A result of "not elevated" on this test has a Negative Predictive Value approaching 100% for determining the absence of acute traumatic intracranial lesions on head CT imaging. (See Appendix C Figure 1 for further information.) This assay is also known as the Analyzer, Traumatic Brain Injury (ATBI) System.
- Key logistical requirements include on-site refrigerated and frozen storage for cartridges and calibration fluids, respectively. Ongoing product development efforts are focused on assessing whether the test cartridges can be stored frozen; simplifying storage requirements and potentially supporting future shelf-life extension activities.

***NOTE**: Related clinical practice guidelines (CPGs): VA/DoD Clinical practice Guideline for Management of Concussion-Mild Traumatic Brain Injury, JTS Traumatic Brain Injury Management and Basic Neurosurgery in the Deployed Environment CPGs*

STRUCTURE TO SUPPORT OPTIMAL USE OF TBI BIOMARKER

To minimize risks associated with use of this novel capability, we recommend a highly controlled rollout of the TBI biomarker. Each theater should restrict interpretation of test results and evacuation decisions to a select number of designated TBI biomarker consultants typically co-located at the Role 3. The **ADVISOR** teleconsultation system may be utilized (Neurosurgery) if a Role 3 consultant is not available. These individuals possess experience in evaluation, triage, and management of TBI in the acute setting and are knowledgeable in the clinical application of TBI biomarker values. The Theater Trauma Medical Director (TMD) is responsible for directing this overall effort but will normally yield clinical decision-making capacity to the neurosurgeon or other neurospecialist if available. The consultant is responsible for supporting the forward provider in execution of the TBI Biomarker Algorithm (Appendix A), use of TBI biomarker results, and disposition of the patient. When a negative TBI biomarker test guides a decision to keep the patient at the forward location, the consultant or TMD should remain engaged with that provider until no longer necessary.

INITIAL EVALUATION

Casualties should initially be evaluated and resuscitated based on JTS guidelines, Advanced Trauma Life Support (ATLS) principles, and Tactical Combat Casualty Care (TCCC) protocols after an acute injury, which may include a potentially concussive event (PCE). PCEs are defined in DoDI 6490.11 and include:

- involvement in a vehicle blast event, collision, or rollover
- presence within 50 meters of a blast (inside or outside)
- a direct blow to the head or witnessed loss of consciousness
- exposure to more than one blast event

The classification of head injury as mild, moderate or severe includes the results of imaging and reports of symptoms for up to 7 days. Thus, for the purposes of initial evaluation in the field, the GCS is the most appropriate initial assessment and has prognostic implications.

Head injured casualties are initially classified according to their GCS score:

- Mild: GCS 13-15
- Moderate: GCS 9-12
- Severe: GCS 3-8

***NOTE**: If the casualty has a GCS of 12 or less (moderate or severe TBI), please refer to the JTS Traumatic Brain Injury Management and Basic Neurosurgery in the Deployed Environment CPG. This Biomarker CPG does not apply.*

If the casualty has no other injuries requiring evacuation, has a GCS of 13 or greater, the MACE2 should be performed. When the TBI whole blood biomarker test is not available, the MACE2 and Enclosure 3 of DoDI 6490.11 should guide initial evaluation in addition to these guidelines. (See Supporting TBI Resources for DoDI 6490.11)

This guidance and algorithm (Appendix A) expands upon the MACE2 and provides initial guidance for locations with access to the new TBI whole blood biomarker test. The TBI whole blood biomarker test should be used in the place of a "structural brain injury" detection device as listed on the MACE 2 under "red flags." It is important to note that the blood biomarker test does not provide a definitive diagnosis of "structural brain injury" but a negative test result can help to rule it out, thus avoiding unnecessary transport and imaging. It should be used and interpreted in accordance with the intended use statement in Appendix B. The MACE2 red flags help to delineate which individuals should undergo assessment for structural brain injury. This CPG seeks to expand upon the MACE 2 to assist providers in understanding the most appropriate population for assessment with the TBI whole blood biomarker.

RISK EVALUATION FOR BRAIN INJURY & INTRACRANIAL HEMORRHAGE

At locations with access to the TBI whole blood biomarker test, a casualty exposed to a PCE should be stratified as high, moderate, or low risk using the Appendix A Algorithm. This is a modification of the MACE2, targeting the population most likely to benefit from the use of the TBI whole blood biomarker.

HIGH RISK: DO NOT DELAY CARE FOR TBI WHOLE BLOOD BIOMARKER

A subset of MACE 2 red flags suggest high risk of brain injury with intracranial hemorrhage. If any one of the following red flag signs or symptoms are present, the casualty should be referred urgently for CT scan.

Do not delay evacuation to obtain TBI whole blood biomarkers.

- Deteriorating levels of consciousness or a drop in post injury GCS score by 2 or greater
- Combativeness or agitated behavior
- 2 or more episodes of vomiting
- Witnessed seizure activity
- Focal neurologic deficits such as pupil asymmetry, facial weakness/asymmetry, weakness or paralysis on one side compared to the other
- Bleeding disorder or therapeutic anti-coagulation with heparin, low molecular weight heparin, warfarin, or novel oral anticoagulants (direct thrombin inhibitors and direct factor Xa inhibitors)

In addition, casualties with evidence suspicious for a penetrating brain injury, depressed skull fracture, or signs of a basilar skull fracture (e.g., raccoon eyes, Battle's sign, otorrhea) should be referred urgently for CT scan.

MODERATE RISK: TEST TBI WB BIOMARKERS BEFORE EVACUATION

This is the target population for TBI whole blood biomarkers. Because of the significant false-positive rate, TBI whole blood biomarker testing will be directed to casualties with moderate risk for intracranial hemorrhage. The goal is to allow symptomatic patients who test negative to forego CT scan and remain in place with activity restrictions (i.e. quarters, bed rest) and treatment of symptoms. There are two important caveats to this group:

1. The concussive event must have taken place within 24 hours of testing.
2. The patient should not have other injuries requiring urgent evacuation (fractures, concern for internal injuries, etc.). In this instance, testing should not hold up transport for more urgent issues but can still be completed.

Casualties without the high-risk red flag signs or symptoms described above but exhibit one or more of the following are appropriate candidates for TBI whole blood biomarker testing.

- Double vision
- Increased restlessness
- < 2 episodes of vomiting
- Subjective weakness or tingling in arms or legs but no clear focal neurological deficit
- Severe, persistent, or worsening headaches
- Age >60 years
- Anti-platelet drugs (such as aspirin or ibuprofen)
- Drug/alcohol intoxication

- Post traumatic amnesia (inability to recall events for 30 or more minutes after injury)
- Worrisome mechanism of injury: high speed motor vehicle collision or rollover; fall from greater than 3ft; or presence within 50m of a blast inside or outside.

Casualties with any of these findings should be evaluated with the TBI whole blood biomarker so long as **the test is performed within 24 hours of the initial head injury.** Then the forward provider contacts the TBI Biomarker Consultant (TMD, neurosurgeon or other neurospecialist, or via **ADVISOR**) and the two come to a decision regarding need for head CT and priority of evacuation. When it is determined that the patient can remain in place with activity restrictions and be treated per the treatment section below, the forward provider and consultant should make a plan to communicate on the patient's progress.

LOW RISK FOR TBI: NO RISK FACTORS PRESENT

If the casualty does not have any of the risk factors described above, the provider should care for the casualty as described in the MACE2. If worsening symptoms develop more than 24 hours after the initial injury, the provider should contact the designated TBI Biomarker Consultant.

Research suggests that some casualties without MACE2 red flags undergo evacuation for head CT despite the MACE2 recommendations. If a provider wishes to obtain a head CT in individuals without the high or moderate risk signs or symptoms described above, a TBI whole blood biomarker test should be performed before referral for a head CT if the casualty is evaluated within 24 hours of injury.

Consultation with the designated TBI Biomarker Consultant is strongly recommended to help determine the urgency of referral and evacuation.

LOCATIONS WITH ON-SITE HEAD CT

The TBI whole blood biomarkers are a new capability both to the military and the civilian sectors. As such, CPGs for the use of TBI whole blood biomarkers have not yet been developed by civilian professional societies. When head CT capabilities are available on-site or evacuation is minimal risk, providers may consider performing both the TBI whole blood biomarkers and head CT to gain additional experience with the TBI whole blood biomarkers in clinical and operational settings.

PRIMARY CONCUSSIVE BLAST

The TBI whole blood biomarker was validated in civilian blunt trauma patients and it is unknown at this time how the test will perform in casualties sustaining TBI from a primary blast wave exposure.

SYMPTOMATIC TREATMENT OF MILD TBI

The hallmark of treatment for service members who sustain an mTBI is relative rest and initial symptom management. Service members with mTBI should be managed in accordance with DoDI 6490.11 and the published DoD Traumatic Brain Injury Center of Excellence MACE 2 and Progressive Return to Activity Clinical Recommendation.

Many casualties with positive (elevated) results with TBI the whole blood biomarker will not have evidence of brain injury or intracranial hemorrhage on head CT but will have brain injury evident on Magnetic Resonance Imaging (MRI).[11] However, a TBI whole blood biomarker result of "elevated" is not FDA approved for the diagnosis of mTBI and should not be used as the sole indicator of mTBI diagnosis; a clinical evaluation of the casualty is necessary to make a diagnosis of mTBI. At this time, it is not known whether casualties with positive (elevated) TBI whole blood biomarkers but no evidence of injury on head CT

should be treated differently from casualties with negative (not elevated) TBI whole blood biomarkers. Therefore, individuals with elevated biomarkers and a negative head CT should be managed as individuals with mTBI as per DoD guidelines cited in the preceding paragraph.

PERFORMANCE IMPROVEMENT (PI) MONITORING

POPULATION OF INTEREST

Casualties exposed to a potentially concussive event.

INTENT (EXPECTED OUTCOMES)

- Documented GCS, neurologic exams, and symptoms for each service member exposed to a potentially concussive event in accordance with the DoD Traumatic Brain Injury Center of Excellence MACE2 and Progressive Return to Activity clinical recommendations.
- TBI whole blood Biomarker performed on moderate risk patients within 24 hours of injury.
- TBI Biomarker Consultant is informed on all positive and negative TBI whole blood Biomarker tests.

PERFORMANCE/ADHERENCE MEASURES

- MACE2 exams documented on all Service Members diagnosed with mTBI
- Documented results of the TBI WB biomarker in the patient's medical record

DATA SOURCES

- Patient Record
- Department of Defense Trauma Registry

SYSTEM REPORTING & FREQUENCY

The above constitutes the minimum criteria for PI monitoring of this CPG. System reporting will be performed annually; additional PI monitoring and system reporting may be performed as needed.

The system review and data analysis will be performed by the JTS Chief and the JTS PI Branch.

RESPONSIBILITIES

It is the trauma team leader's responsibility to ensure familiarity, appropriate compliance and PI monitoring at the local level with this CPG.

REFERENCES

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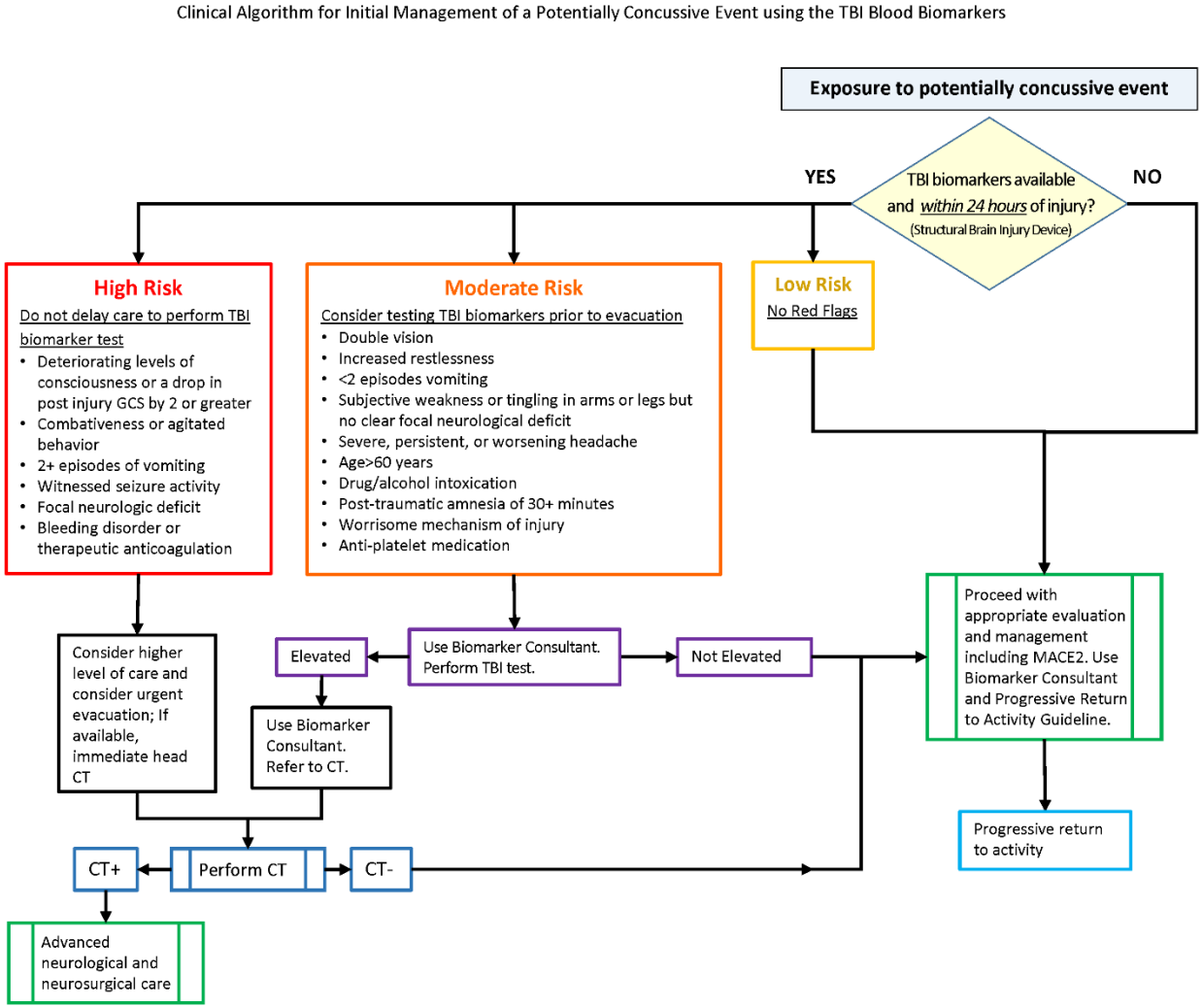
APPENDIX A: CLINICAL ALGORITHM FOR TBI BLOOD BIOMARKERS USE

Clinical Algorithm for Initial Management of a Potentially Concussive Event using the TBI Blood Biomarkers



APPENDIX B: DETAILED INFORMATION ON THE TBI WB BIOMARKER

***NOTE**: Information summarized from the following sources:*

- Abbott Point of Care Inc., 2021, 510(k) Summary: i-STAT TBI Plasma Cartridge with the i-STAT Alinity System (K201778; Approved January 8, 2021). https://www.accessdata.fda.gov/cdrh_docs/pdf20/K201778.pdf
- Abbott Point of Care Inc. 2024, 510(k) Summary: i-STAT TBI Cartridge with the i-STAT Alinity System (K234143; Approved March 27, 2024). https://www.accessdata.fda.gov/cdrh_docs/reviews/K234143.pdf

Capability Name: Analyzer, Traumatic Brain Injury System

Device Name: i-STAT TBI Cartridge with the i-STAT Alinity System

Device and Assay Description:

- Semi-quantitative multiplex immunoassay in ethylenediaminetetraacetic acid (EDTA) anticoagulated WB for:
  - Glial fibrillary acidic protein (GFAP)
  - Ubiquitin carboxyl-terminal hydrolase-L1 (UCH-L1)
  - Cartridge can only be used on the i-STAT Alinity.
  - Test time is approximately 15 minutes
- Will give an error reading if used on i-STAT 1.
- Cartridges require refrigerated storage; calibration/verification and control fluids require freezer for storage.

Whole Blood Assay Reference Range:

- Range derived from N=150 apparently healthy individuals with no history of neurological disease: GFAP: Mean 18.7 pg/mL; Median 18.0 pg/mL; Reference Interval <47-53 pg/mL
- UCH-L1: Mean 89.3 pg/mL; Median 81.5 pg/mL; Reference Interval <87-251 pg/mL

Whole Blood Assay Cut-Off Values and Results:

- Elevated: An elevated result is given if either GFAP OR UCH-L1 is elevated
  - GFAP cut-off: 65 pg/mL
  - UCH-L1 cut-off: 360 pg/mL
- Not elevated: A not elevated result is given if GFAP AND UCH-L1 are below the cut-off
- Not reportable: No result is given if valid results are below the cut-off AND one or more results are not reportable

Plasma and Whole Blood Assay Performance

There were three studies performed as part of the U.S. Food and Drug Administration (FDA) licensure process; two of which were based on plasma samples and one based on whole blood samples.

Study #1: This study was conducted on stored plasma samples and demonstrated performance comparable to the Banyan Biomarkers assay with:

- Sensitivity of 95.8% [95% Confidence Interval (CI) 90.6%, 98.2%]
- Negative predictive value of 99.3% [95%CI 98.5%, 99.7%]
- Specificity of 40.4% [95%CI 38.2%, 42.7%]

(See Appendix C for additional details.)

***Study #2**: This study was conducted on fresh plasma samples and demonstrated comparable sensitivity but lower specificity.*

- Sensitivity of 100.0 [95% CI 88.3%, 100.0%]
- Specificity of 23.7% [95%CI 14.7%, 36.0%]

(See Appendix C for additional details.)

***Study #3:** A prospective, multi-center, observational study was conducted to evaluate the clinical performance of the i-STAT TBI whole blood cartridge in classifying intended use population subjects with suspected mild TBI for the likely absence of acute intracranial lesions visualized by a head CT scan. Testing was performed at 20 external point of care clinical sites across the United States.*

- Clinical Sensitivity 96.5% (273/283) (95% CI 93.6%, 98.1%)
- Clinical Specificity 40.3% (277/687) (95% CI 36.7%, 44.0%)
- Negative Predictive Value (NPV) 96.5% (277/287) (95% CI 93.7%, 98.1%)
- Adjusted NPV at 6% prevalence is 99.4% (95% CI: 99.0%, 99.7%)

Whole Blood TBI Assay Intended Use Statement

The i-STAT TBI test is a panel of in vitro diagnostic immunoassays for the quantitative measurements of glial fibrillary acidic protein (GFAP) and ubiquitin carboxyl-terminal hydrolase L 1 (UCH-L 1) in whole blood and a semi-quantitative interpretation of test results derived from these measurements, using the i-STAT Alinity instrument. The interpretation of test results is used, in conjunction with other clinical information, to aid in the evaluation of patients, 18 years of age or older, presenting with suspected mild traumatic brain injury (Glasgow Coma Scale score 13-15), which may include one of the following four clinical criteria: 1) any period of loss of consciousness, 2) any loss of memory for events immediately before and after the accident, 3) any alteration in mental state at the time of accident, and/or 4) focal neurological deficits, within 24 hours of injury, to assist in determining the need for a CT (computed tomography) scan of the head. A 'Not Elevated' test interpretation is associated with the absence of acute traumatic intracranial lesions visualized on a head CT scan. The test is to be used with venous whole blood collected with EDTA anticoagulant in point of care or clinical laboratory settings by a healthcare professional.

APPENDIX C: SUMMARY OF RESEARCH

Overall Summary of Evidence

Three studies were conducted in support of the FDA licensure request.[1-4] The first study used frozen plasma samples obtained from the study conducted in support of the Banyan Biomarkers TBI Assay.[2,3] This study (n=1901) demonstrated comparable performance to the Banyan Biomarkers assay with a sensitivity of 95.8%, negative predictive value of 99.3%, and specificity of 40.4% [95%CI 38.2%, 42.7%]. There were n=5 false negative test results; none of these were abnormalities requiring surgical intervention.[2]

The second study used a small sample of fresh plasma samples (n=88) obtained from a small subset of patients a study of TBI at Level 1 Trauma Centers who had head CT performed.[3] In this sample the sensitivity and negative predictive value were 100%, but the specificity was lower - 23.7%. The reason for the lower specificity in this population is not clear but may have been due to the small sample size or due to differences in the study population. For example, only 6% of the population in the first study had findings on head CT as compared to this study where 33% had findings on head CT. The positive predictive values for the two studies were comparable after adjustment for the prevalence of positive head CT.

The third study used venous whole blood specimens collected within 24 hours of head injury. The study (n=970) demonstrated comparable performance to the earlier plasma studies, with a sensitivity of 96.5%, negative predictive value of 96.5% (adjusted NPV at 6% prevalence of CT+ was 99.4%), and specificity was 40.3%. There were n=10 false negative results and none of these required surgical intervention.

While the specificity of the assay is low to moderate for brain injury and hemorrhage visible on CT, additional research suggests that many individuals with elevated Glial Fibrillary Acidic Protein using a prototype of the assay have evidence of brain injury on MRI.[5] It is important to note the assay is not FDA approved for this purpose.

Summary of Study #1

Stored frozen plasma samples were obtained from Bazarian, et al study used for approval of Banyan Biomarkers TBI assay, an early version of this assay.[1,2]

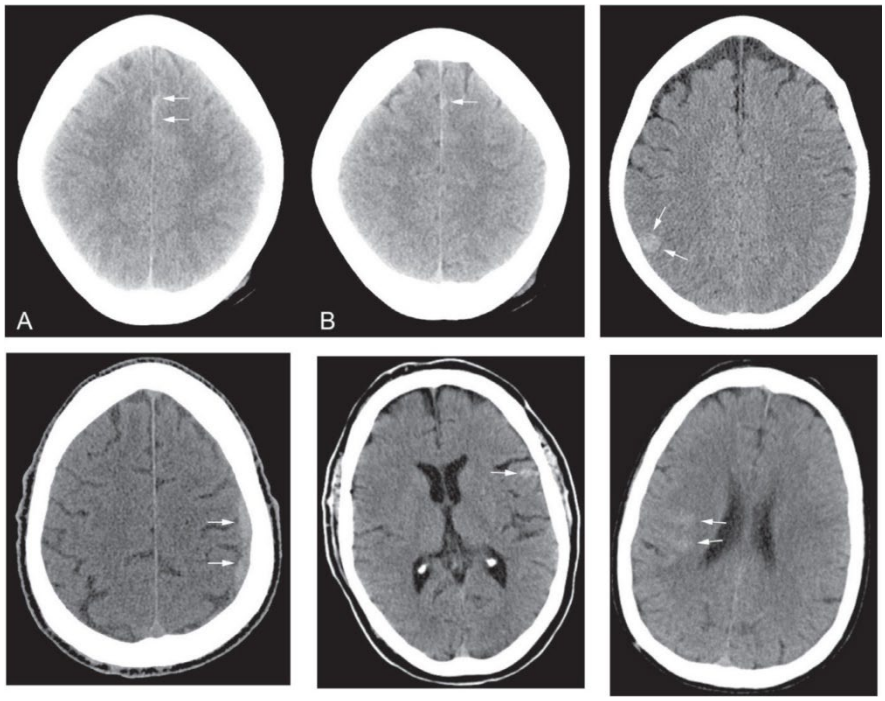
- **Inclusion/Exclusion Criteria**: Adults ≥18 years, presenting to Emergency Department with non-penetrating TBI and GCS 13-15, where the referring provider felt a head CT was indicated, with blood test within 24 hours of injury at 22 sites in the U.S. and Europe
- **The study population** (n=1901) had a median age of 49.0 years and ranged from 18 to 98 years. Over half (56.6%) were male and 70.6% were white and 26.2% were African American.
- Nearly all had a GCS of 15 (94.1%) and around half had Loss of Consciousness (42.2%), Alteration of Consciousness (56.3%), or visible trauma above the clavicle (63.3%). Around one-third reported post-traumatic amnesia (33.0%).
- The median time between injury and blood draw was 3.2 hours with a range of 0.3 to 11.9 hours.

Table 1. Clinical Performance

Clinical Performance			
i-STAT TBI Plasma Test Interpretation	Adjudicated CT Scan Result		Total
	Positive	Negative	
Elevated	115	1061	1176
Not Elevated	5	720	725
Total	120	1781	1901
Clinical Performance Parameters	N=1901	95% Confidence Interval	
Clinical Sensitivity	95.8%	90.6%, 98.2%	
Clinical Specificity	40.4%	38.2%, 42.7%	
Negative Predictive Value (NPV)	99.3%	98.5%, 99.7%	
Positive Predictive Value (PPV)	9.8%	9.2%, 10.2%	
Likelihood Ratio Negative (LRN)	0.10	0.04, 0.23	
Likelihood Ratio Positive (LRP)	1.61	1.15, 1.69	

There were five individuals with false negative results (i.e. not elevated biomarker result and findings on head CT). None of these individuals required surgical intervention. Findings included a small sub-arachnoid hemorrhage, a small subdural hemorrhage and a venous angioma thought to be a congenital anomaly. See Figure 1 for the 5 head CT scans from false negative subjects.

Figure 1. Head CT Scans from false negative subjects



Legend: Non-Contrast CT images. Top Left (subject 1) – Two CT images (A + B) show focal subarachnoid hemorrhage in the anterior, paramedian frontal sulci. Top Right (subject 2) shows a focal area of hyperdensity in the posterior right parietal lobe. On lower slices (not shown), there is a suggestion of some lower attenuation edema which marginates the contusion. Bottom Right (subject 3) shows subdural hemorrhage along the left lateral hemisphere,

overlying the frontal and parietal lobes with minimal local mass effect on the brain parenchyma. Bottom Middle (subject 4) – shows left frontal subarachnoid hemorrhage. Bottom Right (subject 5) shows right temporal subarachnoid hemorrhage. (From Bazarian 2021)

Summary of Study #2

Fresh plasma samples were obtained from four clinical sites of the *Transforming Research and Clinical Knowledge in Traumatic Brain Injury (TRACK-TBI) study* in the United States.[3]

- **Inclusion/Exclusion Criteria**: Adults ≥18 years, presenting to Emergency Department with GCS 13-15, who had a head CT performed at 4 sites in the U.S.
- **The study population** (n=88) had a mean age of 42.5 years and ranged from 18 to 85 years. Nearly three quarters (71.2%) were male.
- Most had a GCS of 15 (81.8%) and over half had loss of consciousness (68.2%), presence of confusion (67.0%), or post-traumatic amnesia (68.2%).
- The median time between injury and blood draw was 4.3 hours with a range of 2.0 to 11.8 hours.

Table 2. Clinical Performance – Supplemental Fresh Specimen Study

Clinical Performance – Supplemental Fresh Specimen Study			
i-STAT TBI Plasma	Adjudicated CT Scan Result		Total
Test Interpretation	Positive	Negative	
Elevated	29	45	74
Not Elevated	0	14	14
Total	29	59	88
Clinical Performance Parameters	**N=88**	**95% Confidence Interval**	
Clinical Sensitivity	100.0%	88.3%, 100.0%	
Clinical Specificity	23.7%	14.7%, 36.0%	
Negative Predictive Value (NPV)*	100.0%	80.2%, 100.0%	
Positive Predictive Value (PPV)*	39.2%	35.9%, 43.4%	
Likelihood Ratio Negative (LRN)	0.00	0.00, 0.50	
Likelihood Ratio Positive (LRP)	1.31	1.14, 1.56	

*Legend: *NPV and PPV estimated at 33.0% prevalence of CT scan positive rate for suspected mild TBI subjects. Adjusted NPV and PPV at 6% prevalence (to be comparable to the pivotal study) are 100.0% (95% CI: 96.9%, 100.0%) and 7.7% (95% CI: 6.8%, 9.1), respectively.*

Summary of Study #3

A prospective, multi-center, observational study was conducted to evaluate the clinical performance of the i-STAT TBI cartridge in classifying intended use population subjects with suspected mild TBI for the likely absence of acute intracranial lesions visualized by a head CT scan. Venous whole blood specimens were collected in K2EDTA within 24 hours of the head injury.[4]

- **Inclusion/Exclusion Criteria**: Adults ≥18 years, presenting to Emergency Department with non-penetrating TBI and GCS 13-15, who had a head CT ordered as part of their standard of care, with blood test within 24 hours of injury at 20 *Transforming Research and Clinical Knowledge in Traumatic Brain Injury (TRACK-TBI)* sites.
- **The study population** (n=970) had a median age of 46.0 years and ranged from 18 to 97 years. Over half (64.0%) were male and 68.6% were white and 17.7% were African American.
- A majority had a GCS of 15 (78.6%) and about two thirds had Loss of Consciousness (69.6%), Alteration of Consciousness (72.1%), visible trauma above the clavicle (65.6%) and post-traumatic amnesia (62.4%).
- The median time between injury and blood draw was 8.1 hours with a range of 1.5 to 24 hours.

Table 3. Clinical Performance

Clinical Performance			
i-STAT TBI Plasma Test Interpretation	Adjudicated CT Scan Result		Total
	Positive	Negative	
Elevated	273	410	683
Not Elevated	10	277	287
Total	283	687	970
Clinical Performance Parameters	N=970	95% Confidence Interval	
Clinical Sensitivity	96.5%	(93.6%, 98.1%)†	
Clinical Specificity	40.3%	(36.7%, 44.0%)†	
Negative Predictive Value (NPV)*	96.5%	(93.7%, 98.1%)‡	
Positive Predictive Value (PPV)	40%	(38.4%, 41.5%)‡	
Likelihood Ratio Negative (LRN)	0.09	(0.05, 0.16)§	
Likelihood Ratio Positive (LRP)	1.62	(1.52, 1.73)§	

*Legend: * Adjusted NPV at 6% prevalence is 99.4% (95% CI: 99.0%, 99.7%). †95% confidence intervals are calculated using the Wilson score method for a binomial portion (see CLSI EP12-Ed3). ‡95% confidence intervals for predictive values are calculated based on the confidence intervals of the corresponding likelihood ratios §95% confidence intervals are calculated using asymptotic method for a ratio of two binomial proportion*

Summary of GFAP/MRI Study

Yue JK, Yuh EL, Korley FK, et al. Association between plasma GFAP concentrations and MRI abnormalities in patients with CT-negative traumatic brain injury in the TRACK-TBI cohort: a prospective multicentre study. Lancet Neurol. 2019;18(10): 953-961. https://doi.org/10.1016/s1474-4422(19)30282-0

The study enrolled adults with GCS 13-15 between 2014-2018 at 18 participating Level 1 U.S. trauma centers who presented within 24 hours of injury and had a head CT as well as an MRI within 7-18 days post injury.[5]

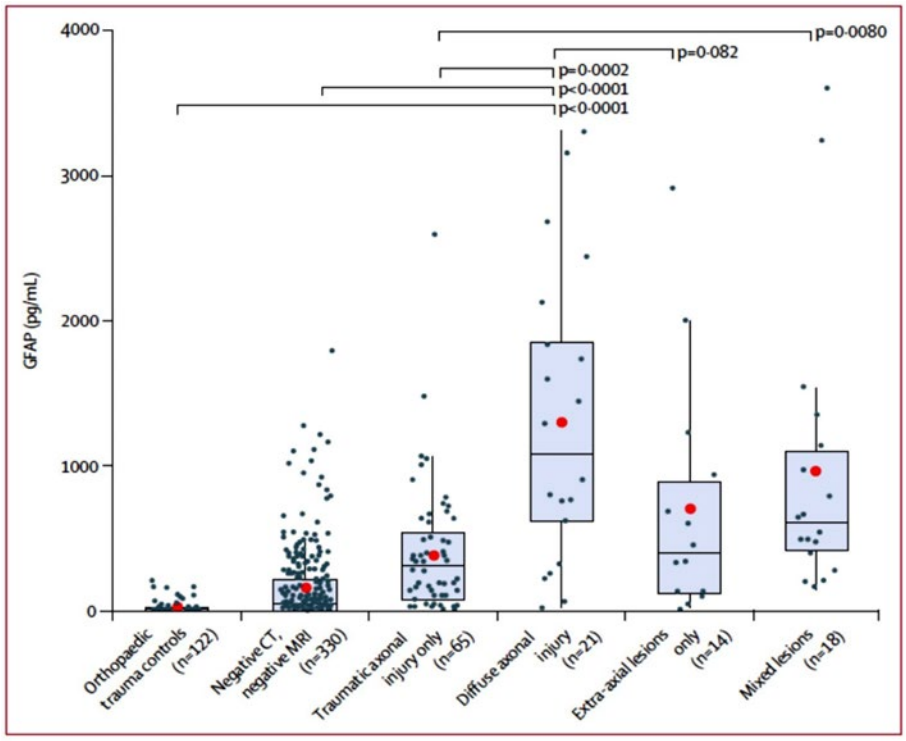
- Of 1375 individuals enrolled in TRACK-TBI study, 794 had negative head CT and a GCS of 13-15 of whom 450 had an MRI scan performed within 7-18 days. Of these, 27% had positive MRI.
- Individuals with positive head CT had the highest GFAP levels (Median 786.0 pg/mL); followed by those with negative head CT and positive MRI (Median 414.4 pg/mL); followed by those with negative head CT and negative MRI (Median 74.0 pg/mL). Of note, healthy (Median 8.0 pg/mL) and orthopedic trauma (Median 13.1 pg/mL) controls had significantly lower GFAP levels (Table 4/Figure 2).
- From a pathophysiologic perspective, elevated GFAP levels were noted in traumatic axonal injury, diffuse axonal injury, extra-axial and mixed lesions (See Figure 2).
- The sensitivity and specificity of GFAP levels for positive MRI is listed in Table 5. Of note, GFAP levels greater than 282.70 pg/mL has a specificity of 80.3% for positive MRI.

Table 4. WB GFAP concentrations by imaging modality and findings

Plasma GFAP concentrations by imaging modality and findings					
	No of patients	WB GFAP concentrations (pg/mL)			
		Mean (SD)	Median (25-75th percentile)	Range	P value
Positive CT	199	1400.9 (1598.6)	786.0 (357.0-1863.3)	0-9409.7	<0.0001*
Negative CT	450	308.0 (530.5)	110.3 (22.7-352.3)	0-4095.1	..
Negative CT & positive MRI	120	692.2 (827.6)	414.4 (139.3-813.4)	5.2-4095.1	<0.00001†
Negative CT & negative MRI	330	168.3 (250.9)	74.0 (17.5-214.4)	0-1864.5	..
Orthopaedic trauma controls	122	23.7 (37.2)	13.1 (6.9-20.0)	0-216.8	<0.0001‡∫
Healthy controls	209	11.0 (12.7)	8.0 (3.0-14.0)	0-98.0	<0.0001‡∫

*GFAP=glial fibrillary acidic protein. P values were calculated from the Wilcoxon rank sum test for the comparisons, which compares the distributions of the two groups. *Compared with patients with negative CT. †Compared with patients with negative CT and negative MRI findings. ‡Compared with patients with negative CT and positive MRI findings. ∫ Compared with patients with negative CT and negative MRI findings.*

Figure 2. GFAP concentration by MRI pathology



The red dot signifies mean WB GFAP concentration while boxplots provide range, median, and 25-75th percentiles. Individual dot values are plotted for reference. The Dunn Kruskal-Wallis test for comparisons among different MRI lesion types with a Benjamin-Hochberg correction for multiple comparisons[23] showed that GFAP concentrations are significantly higher in patients with isolated diffuse axonal injury than in those with isolated traumatic axonal injury. Separate Wilcoxon rank sum tests also showed significantly higher GFAP concentrations in patients with isolated diffuse axonal injury than in patients with negative CT and negative MRI findings, and orthopaedic trauma controls. P values are displayed for relevant comparisons. Two patients with isolated intracerebral contusions (GFAP 14.9 pg/mL, 285 4 pg/mL were not included as boxplot. GFAP=glial fibrillary acidic protein.

Table 5. Cutoff concentrations of WB GFAP to predict MRI-positive versus MRI-negative findings in patients with negative CT

	Sensitivity	Specificity	NPV	PPV
4.40 pg/mL	1.000 (1.000-1.000)	0.024 (0.009-0.042)	1.000 (1.000-1.000)	0.271 (0.268-0275)
12.95 pg/mL	0.958 (0.925-0.992)	0.188 (0.148-0.230)	0.925 (0.863-0.981)	0.300 (0.287-0.313)
25.15 pg/mL	0.908 (0.850-0.958)	0.333 (0.288-0.388)	0.910 (0.861-0.957)	0.332 (0.312-0.354)
71.95 pg/mL	0.825 (0.750-0.892)	0.494 (0.442-0.549)	0.888 (0.845-0.924)	0.373 (0.344-0.407)
282.70 pg/mL	0.642 (0.558-0.733)	0.803 (0.758-0.842)	0.861 (0.832-0.890)	0.543 (0.482-0.603)
848.75 pg/mL	0.233 (0.158-0.308)	0.964 (0.942-0.982)	0.775 (0.760-0.793)	0.698 (0.555-0.842)

The k-fold cross validation method was used to select the optimal cutoffs for predicting MRI-positive versus MRI-negative findings in patients with negative CT based on the criteria of adjusted NPV above the level of 0·96, 0·94, 0·92, and 0·90, 0·85, and 0·80, in accordance with data standards for clinical laboratory assays set by the manufacturer. The prevalence of positive MRI scans among patients with negative CT scans was estimated to be 0·27 on the basis of the sample rate to calculate the adjusted NPV.1000 bootstraps were conducted to determine the optimal cutoffs using the median from each run. The optimal cutoff thresholds were than applied to the full data to calculate the corresponding sensitivity, specificity, NPV, and PPV. GFAP=glial fibrillary acidic protein. NPV=negative predictive value. PPV=positive predictive value.

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APPENDIX D: CLASS VIII PLACEHOLDER

APPENDIX E: TELEMEDICINE / TELECONSULTATION

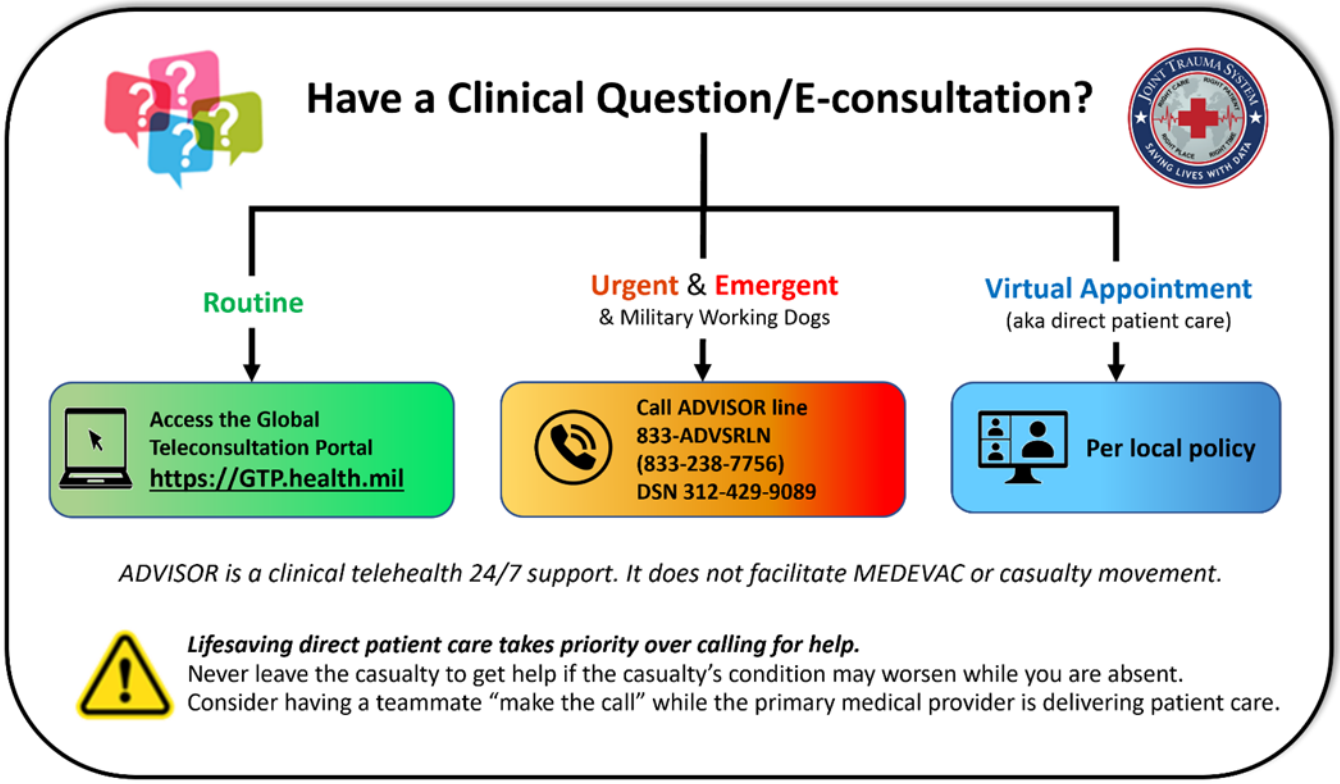

Illustration by Raymond Samonte

GTP: https://GTP.health.mil

APPENDIX F: INFORMATION REGARDING OFF-LABEL USES IN CPGS

PURPOSE

The purpose of this Appendix is to ensure an understanding of DoD policy and practice regarding inclusion in CPGs of "off-label" uses of U.S. Food and Drug Administration (FDA)–approved products. This applies to off-label uses with patients who are armed forces members.

BACKGROUND

Unapproved (i.e. "off-label") uses of FDA-approved products are extremely common in American medicine and are usually not subject to any special regulations. However, under Federal law, in some circumstances, unapproved uses of approved drugs are subject to FDA regulations governing "investigational new drugs." These circumstances include such uses as part of clinical trials, and in the military context, command required, unapproved uses. Some command requested unapproved uses may also be subject to special regulations.

ADDITIONAL INFORMATION REGARDING OFF-LABEL USES IN CPGS

The inclusion in CPGs of off-label uses is not a clinical trial, nor is it a command request or requirement. Further, it does not imply that the Military Health System requires that use by DoD health care practitioners or considers it to be the "standard of care." Rather, the inclusion in CPGs of off-label uses is to inform the clinical judgment of the responsible health care practitioner by providing information regarding potential risks and benefits of treatment alternatives. The decision is for the clinical judgment of the responsible health care practitioner within the practitioner-patient relationship.

ADDITIONAL PROCEDURES

Balanced Discussion

Consistent with this purpose, CPG discussions of off-label uses specifically state that they are uses not approved by the FDA. Further, such discussions are balanced in the presentation of appropriate clinical study data, including any such data that suggest caution in the use of the product and specifically including any FDA-issued warnings.

Quality Assurance Monitoring

With respect to such off-label uses, DoD procedure is to maintain a regular system of quality assurance monitoring of outcomes and known potential adverse events. For this reason, the importance of accurate clinical records is underscored.

Information to Patients

Good clinical practice includes the provision of appropriate information to patients. Each CPG discussing an unusual off-label use will address the issue of information to patients. When practicable, consideration will be given to including in an appendix an appropriate information sheet for distribution to patients, whether before or after use of the product. Information to patients should address in plain language: a) that the use is not approved by the FDA; b) the reasons why a DoD health care practitioner would decide to use the product for this purpose; and c) the potential risks associated with such use.