Human Bocavirus as a Possible Contributor to Respiratory Disease in the Georgian Military Population

*Nino Akhvlediani, MD[1]; Shannon Walls, MS[2]; Nabil H. Latif, DVM[3]; Nino Markhvashvili, MD[4]; Neli Javakhishvili, MD[5]; Nino Mitaishvili, PhD[6]; David Barliani, MD[7]; Kalei Hering[8]; Michael A. Washington, PhD[9]**

ABSTRACT

The coronavirus disease 2019 (COVID-19) pandemic has demonstrated that new and devastating respiratory pathogens can emerge without warning. It is therefore imperative that Special Operations medical personnel be aware of the presence of emerging pathogens within their area of operation. Human bocavirus (HBoV) is a newly described member of a family of viruses known as the Parvovirinae that are often associated with acute respiratory illness. The presence of HBoV in the country of Georgia has not been previously reported. Nasal and throat swabs were collected from 95 symptomatic members of the Georgian military. HBoV was detected in 11 of them (12%). To our knowledge, this is the first report of HBoV infection in the country of Georgia. This finding may have a significant impact on members of the Special Operations community who train in Georgia as more data concerning the transmission, pathogenesis, and treatment of HBoV are accumulated and the role of HBoV in human disease is more clearly defined.

KEYWORDS: *coronavirus disease 2019; COVID-19; respiratory pathogens; bocavirus; human bocavirus*

Introduction

HBoV was discovered in 2005.[1] The name bocavirus comes from the combination of the first two letters of "bovine parvovirus" with the first two letters of "canine minute virus." These viruses primarily infect domestic animals, resulting in acute gastrointestinal dysfunction and stillbirth. The name bocavirus was chosen because of similarities in the genetic sequence and genomic organization between the newly described HBoV and these two close relatives.[1] To date, HBoV has primarily been detected as the cause of respiratory infection in young children.[2] However, it has also been detected in adults as a component of multipathogen synergistic infections. HBoV is most often found in nasopharyngeal secretions, in whole blood, and in the serum of patients with upper and lower respiratory tract infections. However, it has also been detected in fecal specimens derived from patients presenting with acute gastroenteritis. Although the exact cause of the gastroenteritis in these patients has not been determined, this finding indicates that HBoV may be involved in the pathogenesis of gastrointestinal illness in humans, just as bovine parvovirus and canine minute virus are involved in the pathogenesis of gastrointestinal illness in domestic animals.[3,4] HBoV is distributed worldwide, and it has been detected in clinical material derived from both children and adults in Europe, Asia, the Americas, Africa, and Australia.[3] The transmission of HBoV infection is seasonal; it has been found to occur at a constant low level throughout the year, but detections tend to peak during late winter and early spring.[5]

The mechanism of HBoV pathogenesis has not been fully elucidated, and it is not known whether the various disease states that have been associated with this virus result from the virus itself or from coinfection with other pathogens. Indeed, it is unclear whether HBoV functions as a pathogen or a passenger in most of the cases in which it has been detected.[6] This lack of granularity regarding the role of this virus in the pathogenicity of infectious disease is because an appropriate animal model capable of sustaining the growth of HBoV has not been developed, and the virus is notoriously difficult to culture in the laboratory environment.[7] Furthermore, HBoV infection is often accompanied by coinfection with bacteria and other viruses. Notably, coinfection has been detected in up to 83% of respiratory samples in which HBoV was present.[8] It is possible that the virus may be a passenger in some cases and a pathogen in other cases, but there are currently no methods available to distinguish between these two possibilities.[9] Further research will be required to resolve these issues, and the development of robust animal and cell culture models will be a prerequisite for the determination of the role of HBoV in the development of disease.

Despite the current limitations, a variety of signs and symptoms has been described in patients with HBoV infection, and these have been used to generate a standardized description of the results of infection with this virus.[10] The symptoms of HBoV infection include rhinitis, pharyngitis, cough, dyspnea, wheezing, pneumonia, acute otitis media, fever, nausea, vomiting, and diarrhea. Many of these potential manifestations have not been systematically explored, and many have been questioned because of the detection of high levels of coinfection in symptomatic subjects and high HBoV detection rates in asymptomatic subjects.[4,5] It is important to note that severe cases of HBoV infection without coinfection have also

*Correspondence to michael.washington@westpoint.edu

[1]**Dr Akhvlediani**, [2]**Mr Walls**, and [3]**Dr Latif** are affiliated with the United States Army Medical Research Directorate–Georgia, Tbilisi, Georgia. [4]**Dr Mitaishvili** is affiliated with the United States Army Medical Research Directorate–Georgia, Tbilisi, Georgia, and with the Military Hospital of the Ministry of Defense of Georgia, Gori, Georgia. [5]**Dr Javakhishvili** and [6]**Dr Barliani** are affiliated with the Military Hospital of the Ministry of Defense of Georgia, Gori, Georgia. [7]**Ms Hering** and [8]**Dr Washington** are affiliated with the Department of Chemistry and Life Science, United States Military Academy, West Point, NY.

been described. Indeed, a fatal case of an immunocompetent elderly patient resulting from primary viral pneumonia caused by HBoV has been reported in Japan.[11] Such reports underscore the need for military medical providers to be familiar with HBoV and suggest that the incorporation of an assay to detect this virus into the protocols for the diagnosis of cases of acute respiratory infection is warranted.[12]

At present, HBoV is mainly detected using molecular techniques such as polymerase chain reaction (PCR) and reverse transcriptase–PCR. This method detects the presence of viral genetic material and is indicative of a current infection. Serologic methods can also be used to detect the presence of antibodies to the virus, but this method is not routine because of the complexity of the procedure and lack of commercial kits and standardized procedures.[13] Further, serologic testing is indicative only of previous exposure to the virus and cannot be used to identify the presence of an active infection. Molecular testing for the presence of HBoV in a recent study in Spain involved the analysis of more than 3000 nasopharyngeal aspirates and revealed the presence of this virus in 319 patients (9.9%). Eighty of those tests (25%) detected HBoV as a single pathogen, and 239 (75%) detected HBoV as a coinfection with other viruses.[14] A similar study conducted in central Russia involved the screening of more than 5500 stool samples of children with gastroenteritis for HBoV. Viral DNA consistent with HBoV was found in 1.2% of the samples. In addition, it was found that coinfection was most frequently detected with rotavirus A and norovirus GII, both of which are recognized as common causes of acute gastroenteritis.[15] Significantly, HBoV has been detected in healthy human blood, including blood collected from healthy blood donors. This is concerning because of the heavy reliance of the military healthcare system on donated blood for the stabilization and treatment of combat casualties. Indeed, one study revealed that of 300 donor blood samples that were screened for HBoV, 21 were positive.[16] It is important to note that, at present, there is no restriction on the use HBoV positive blood or blood products for patient care and that testing for HBoV is not a routine practice in most blood banks and blood donor centers.[17]

There is currently no information about HBoV prevalence in the country of Georgia. This is concerning, given that respiratory viruses are known to have a negative impact on military operational readiness.[18] In this sentinel surveillance study, we evaluated military personnel who were referred to the Georgian Military Hospital with influenza-like symptoms for the presence of HBoV. In addition, we screened all samples with a panel of common respiratory viruses. HBoV was detected in 11 of 95 respiratory samples (12%) that were collected for this study. This is the first detection of HBoV in the country of Georgia, and the results indicate that this virus is currently circulating among the military population and that it is most likely circulating among the Georgian population.

Methods

Study Site

The Georgian Military Hospital, located in the city of Gori, was selected as the study site. This hospital provides medical service for Georgian military personnel from throughout the country. In case of an outbreak of acute respiratory illness (ARI), the infectious disease department of the Military Hospital manages both inpatient and outpatient cases.

Study Population

Military personnel aged ≥18 years who were referred to the infectious diseases department of the Military Hospital with signs or symptoms of acute respiratory infection were offered to voluntarily participate in the study. Each study subject gave a written informed consent. The study was approved by the institutional review boards of the Military Hospital and the Walter Reed Army Institute of Research. Patients <18 years old and illiterate subjects were excluded (the current literacy rate in Georgia is 99%[19]).

Study Procedure

Participants were asked to complete a standardized epidemiologic questionnaire at the time of enrollment. The questionnaire included demographic data (e.g., gender, travel history, contact with animals, date of illness) and clinical data (e.g., signs and symptoms, duration of illness, secondary complications). This study did not alter the diagnosis or treatment procedures regularly conducted at the Military Hospital.

One nasal swab and one throat swab were obtained at the time of enrollment. Once a week, samples were transferred to the US Army Medical Research Directorate in Georgia lab for testing and archiving. This laboratory is located in Tbilisi, at the Lugar Center for Public Health Research. Before testing, all samples were at stored at –80°C (–112°F). Both nasal and throat swab samples were tested using a multiplex PCR platform developed by Fast-Track Diagnostics (Alzette, Luxembourg), which detects the following pathogens: influenza A, H1N1, influenza B, rhinovirus, coronavirus NL63, 229E, OC43, HKU1, parainfluenza types 1, 2, 3, and 4, human metapneumovirus A/B, respiratory syncytial virus A/B, adenovirus, enterovirus, parechovirus, and HBoV.[20] All laboratory testing was performed following the manufacturer's instructions for the use of the Fast-Track platform.

Results

To determine whether HBoV is circulating in the military population in the country of Georgia, a cohort of military personnel presenting to the Military Hospital with acute respiratory illness was enrolled into the study. Between January and December 2016, 95 subjects were enrolled. Only one subject was a female. The mean age of the subjects was 24 years. All patients presented with flu-like symptoms. The highest detected temperature was 39.8°C (103.6°F), and 92% of subjects had a fever (>38°C [>100.4°F]) at the time of presentation. None of the subjects had been vaccinated against influenza, and 30% of the subjects required hospitalization.

Nasal and throat swabs were collected from each subject. The presence of DNA consistent with HBoV or a panel of common respiratory viruses was detected using a multiplex PCR assay. HBoV was found to be positive in 11 of the 95 subjects (12%). Both nasal and throat swabs were positive in only one patient (1%). The nasal swab alone was found to be positive for six subjects (6%), and only the throat swab was positive in four subjects (4%). Subtypes of the virus were not defined.

The symptoms experienced by subjects with positive HBoV detections did not differ markedly from those of the total study population (Table 1). Some differences included the presence of cough, runny eyes, ringing in the ears, malaise, dizziness, and sinus pain, all of which were found to be slightly

TABLE 1 *Frequency of Signs and Symptoms in HBoV-Positive Group (n = 11) in Comparison With the General Study Group (n = 95)*

Signs/Symptoms	Number and Percentage in Bocavirus-Positive Subjects	Number and Percentage in Total Study Population
Cough	11 (100%)	87 (92%)
Earache/trouble hearing	1 (0.9%)	7 (7%)
Has the patient been hospitalized?	6 (55%)	29 (30%)
Stiff neck	1 (0.9%)	13 (10%)
Eye pain	6 (55%)	49 (52%)
Muscle aches	8 (73%)	69 (73%)
Runny eyes	2 (18%)	7 (7%)
Wheezing	0 (0%)	0 (0%)
Hoarseness	5 (45)	21 (22%)
Trouble breathing	1 (0.9%)	2 (2%)
Ringing in ears	3 (27%)	7 (7%)
Fever	11 (100%)	93 (98%)
Malaise	9 (82%)	57 (60%)
Dizziness	7 (64%)	53 (56%)
Sinus pain	6 (55%)	26 (27%)
Sore throat	8 (73%)	73 (77%)
Runny/stuffy nose	6 (55%)	50 (53%)
Headache	11 (100%)	87 (92%)
Photophobia	2 (18%)	17 (18%)
Pneumonia	4 (36%)	6 (6%)
Vaccinated against influenza	0 (0%)	0 (0%)

TABLE 2 *Rates of HBoV Coinfection With Common Respiratory Viruses for Each of the 11 Detections of HBoV, in Comparison With the Total Number of Detections of Each Virus in the Total Study Population of 95 Subjects*

Virus	Number and Percentage in Bocavirus-Positive Subjects (n = 11)	Number and Percentage in Total Study Population (n = 95)
Influenza A	2 (18%)	43 (45%)
HPIV1	9 (82%)	12 (13%)
HPIV3	2 (18%)	5 (5%)
HPIV4	1 (9%)	1 (1%)
Respiratory syncytial virus	1 (9%)	1 (1%)
Enteroviruses	1 (9%)	0 (0%)
Rhinovirus	1 (9%)	10 (11%)
Human metapneumovirus A/B	7 (64%)	10 (11%)

HPIV = human parainfluenza virus.

increased in subjects that were coinfected with HBoV in comparison with the total study population. One notable difference between the HBoV-positive group and the general study population was the presence of pneumonia, which was more common in the HBoV-positive group than in the general study group (33% vs 6%).

Coinfections were detected in every HBoV-positive case. Coinfecting viruses included some of the most common respiratory pathogens, including influenza, human parainfluenza virus, respiratory syncytial virus, human metapneumovirus, enterovirus, and rhinovirus. The highest rate of coinfection was detected with human parainfluenza virus 1, which was detected in nine cases of coinfection, followed by human metapneumovirus A/B, which was detected in seven cases of coinfection (Table 2).

Discussion

HBoV is a newly discovered virus and, to our knowledge, it has not previously been detected in the country of Georgia. In this study, we detected 11 positive cases of HBoV of a total 95 tested subjects who reported to the Georgian Military Hospital with acute respiratory disease. Consistent with existing literature, all the positive cases were also positive for one or more coinfecting respiratory pathogens. The role of HBoV in the pathogenesis of respiratory disease is not fully understood. Our data support the notion that this virus often coexists with other respiratory viruses and that it may participate in the pathologic process.

The military population of Georgia predominantly consists of young adults, and because HBoV is more common in children, it is possible that younger people are at a higher risk for coinfection with this virus than are older people. A possible positive correlation was detected between the presence of HBoV and the development of pneumonia. This finding may indicate that HBoV tends to increase the severity of acute respiratory illness, a phenomenon that might also be reflected in the increases in the presence of cough, runny eyes, ringing in the ears, malaise, dizziness, and sinus pain that was also observed. Although the mechanism of HBoV pathogenesis is not currently known, it is possible that HBoV pathogenesis synergizes with the pathologic processes of coinfecting viruses, thus leading to an enhancement of the overall pathologic process and an increase in disease symptomology.

Significantly, the symptoms and the seasonality of HBoV overlap with those of the novel SARS-CoV-2 virus that is the causative agent of the COVID-19 pandemic.[21] It is therefore possible that HBoV infection can confound the diagnosis of respiratory disease in Georgia. This may be especially true in regions of the country that do not have access to a robust clinical laboratory infrastructure and molecular diagnostics.

There are several limitations to this study. The major limitation is that this was purely a molecular study with a small sample size and that the virus was not isolated or directly observed; it is therefore impossible to determine whether the positive PCR results were indicative of active infections or whether they resulted from the amplification of the fragmented DNA released from inactive virus. Another limitation is that Koch's postulates for HBoV have not been fulfilled, and because the role of this virus in respiratory disease has not been completely elucidated, it is impossible to determine whether the respiratory symptoms identified in this study are a direct result of the HBoV infection of whether they result primarily from the coinfecting virus or bacteria. A further limitation is that the age range of the study population does not match the typical age of Special Operations personnel. It is hoped that, despite these limitations, the data presented in this report will serve to inform members of the military medical community about the presence of HBoV in Georgia. It is also hoped that this

data may stimulate further investigation into the natural history and the transmission patterns of this emerging pathogen. While HBoV may not be a highly virulent or a highly transmissible pathogen at present, previous studies have indicated that it has the potential to become a serious cause for concern. One of these studies has reported that different strains of HBoV can swap and recombine genetic material.[22] This "gene swapping" may lead to increasing awareness that new or more virulent strains can emerge.[23] There is also evidence that this virus can interact with other viral pathogens in the host, possibly leading to increased pathogenicity.[22]

Georgia was the largest non-NATO contributor to the Resolute Support mission in Afghanistan. In addition, Georgia has participated in numerous NATO and European Union peacekeeping operations, and it deployed several combat infantry brigades to assist US forces in Iraq. It is anticipated that US military personnel will continue to work side by side with their Georgian counterparts in Georgia and throughout the world. Understanding the impact of HBoV and other emerging pathogens on the Georgian military will allow the medical personnel of the US Special Operations community to better assist in the diagnosis and treatment of both Georgian and US military personnel. It is therefore recommended that military medical personnel operating in Georgia and throughout the Caucasus region become familiar with HBoV and that HBoV be considered in the differential diagnosis of acute respiratory infection. This may be especially important in cases where the symptoms are more severe than expected during the winter and early spring months and when there is a possibility for coinfection or confounding with SARS-CoV-2. It is also recommended that surveillance efforts be directed toward determining the actual burden of HBoV in the Georgian population and that research be undertaken to determine the mechanisms of HBoV pathogenesis, so that informed decisions can be made regarding the development and selection of diagnostic and treatment protocols capable of mitigating the threat of this agent to military personnel in Georgia.

Disclaimers

The views expressed in this document are those of the authors and do not reflect the official policy or position of the United States Army, the United States Military Academy or the United States Department of Defense.

Disclosure

The authors have no affiliations with or involvement in any organization or entity with any financial interest in the material discussed in this manuscript.

Funding

This work was financially supported by the Armed Forces Health Surveillance Branch, Global Emerging Infections Surveillance and Response System (AFHSB-GEIS), Silver Spring, Maryland, USA (ProMIS ID: 20160280193, Funding Year: 2016).

References

1. **Guido M, Tumolo MR, Verri T, et al.** Human bocavirus: current knowledge and future challenges. *World J Gastroenterol*. 2016;22(39):8684–8697.
2. **Verbeke V, Reynders M, Floré K, et al.** Human bocavirus infection in Belgian children with respiratory tract disease. *Arch Virol*. 2019;164(12):2919–2930.
3. **Principi N, Piralla A, Zampiero A, et al.** Bocavirus infection in otherwise healthy children with respiratory disease. *PLoS One*. 2015;10(8):e0135640. Published online 2015 August 12.
4. **Jartti T, Hedman K, Jartti L, Ruuskanen O, Allander T, Söderlund-Venermo M.** Human bocavirus: the first 5 years. *Rev Med Virol*. 2012;22(1):46–64.
5. **Kesebir D, Vazquez M, Weibel C, et al.** Human bocavirus infection in young children in the United States: molecular epidemiological profile and clinical characteristics of a newly emerging respiratory virus. *J Infect Dis*. 2006;194(9):1276–1282.
6. **Christensen A, Kesti O, Elenius V, et al.** Human bocaviruses and paediatric infections. *Lancet Child Adolesc Health*. 2019;3(6):418–426.
7. **Schildgen O, Müller A, Allander T, et al.** Human bocavirus: passenger or pathogen in acute respiratory tract infections? *Clin Microbiol Rev*. 2008;21(2):291–304.
8. **Wang K, Wang W, Yan H, et al.** Correlation between bocavirus infection and humoral response, and co-infection with other respiratory viruses in children with acute respiratory infection. *J Clin Virol*. 2010;47(2):148–155.
9. **Chow BD, Esper FP.** The human bocaviruses: a review and discussion of their role in infection. *Clin Lab Med*. 2009;29(4):695–713.
10. **Lee HN, Koo HJ, Kim SH, Choi SH, Sung H, Do KH.** Human bocavirus infection in adults: clinical features and radiological findings. *Kor J Radiol*. 2019;20(7):1226–1235.
11. **Ishiguro T, Hirota S, Kobayashi Y, et al.** Fatal primary human bocavirus pneumonia in an immunocompetent adult. *Intern Med*. 2020;59(3):421–424.
12. **Hang VTT, Ny NTH, Phuc TM, et al.** Evaluation of the Luminex xTAG Respiratory Viral Panel FAST v2 assay for detection of multiple respiratory viral pathogens in nasal and throat swabs in Vietnam. Version 2. *Wellcome Open Res*. 2017;2:80. Published online 2018 Apr 30.
13. **Xu M, Arku B, Jartti T, et al.** Comparative diagnosis of human bocavirus 1 respiratory infection with messenger RNA reverse-transcription polymerase chain reaction (PCR), DNA quantitative PCR, and serology. *J Infect Dis*. 2017;215(10):1551–1557.
14. **Calvo C, García-García ML, Pozo F, Carballo D, Martínez-Monteserín E, Casas I.** Infections and coinfections by respiratory human bocavirus during eight seasons in hospitalized children. *J Med Virol*. 2016;88(12):2052–2058.
15. **Tymentsev A, Tikunov A, Zhirakovskaia E, et al.** Human bocavirus in hospitalized children with acute gastroenteritis in Russia from 2010 to 2012. *Infect Genet Evol*. 2016;37:143–149.
16. **Abdel-Moneim AS, Mahfouz ME, Zytouni DM.** Detection of human bocavirus in Saudi healthy blood donors. *PLoS One*. 2018;13(2):e0193594. Published online 2018 February 28.
17. **Vo MT, Bruhn R, Kaidarova Z, Custer BS, Murphy EL, Bloch EM.** A retrospective analysis of false-positive infectious screening results in blood donors. *Transfusion*. 2016;56(2):457–465.
18. **Clemmons NS, McCormic ZD, Gaydos JC, Hawksworth AW, Jordan NN.** Acute respiratory disease in US Army trainees 3 years after reintroduction of adenovirus vaccine 1. *Emerg Infect Dis*. 2017;23(1):95–98.
19. Georgia Adult Literacy Rate. https://knoema.com/atlas/Georgia/topics/Education/Literacy/Adult-literacy-rate. Retrieved January 16, 2020.
20. **Salez N, Vabret A, Leruez-Ville M, et al.** Evaluation of four commercial multiplex molecular tests for the diagnosis of acute respiratory infections. *PLoS One*. 2015;10(6):e0130378. Published online 2015 June 24.
21. **Kissler SM, Tedijanto C, Goldstein E, Grad YH, Lipsitch M.** Projecting the transmission dynamics of SARS-CoV-2 through the postpandemic period. *Science*. 2020;368(6493):860–868.
22. **Brockmann M, Windisch W, Hedman K, et al.** HBoV-1 in pleura of an adult patient in Cologne, Germany. *JMM Case Rep*. 2014;1(3):e003657.
23. **Zhao M, Zhu R, Qian Y, et al.** Prevalence analysis of different human bocavirus genotypes in pediatric patients revealed intragenotype recombination. *Infect Genet Evol*. 2014;27:382–388.

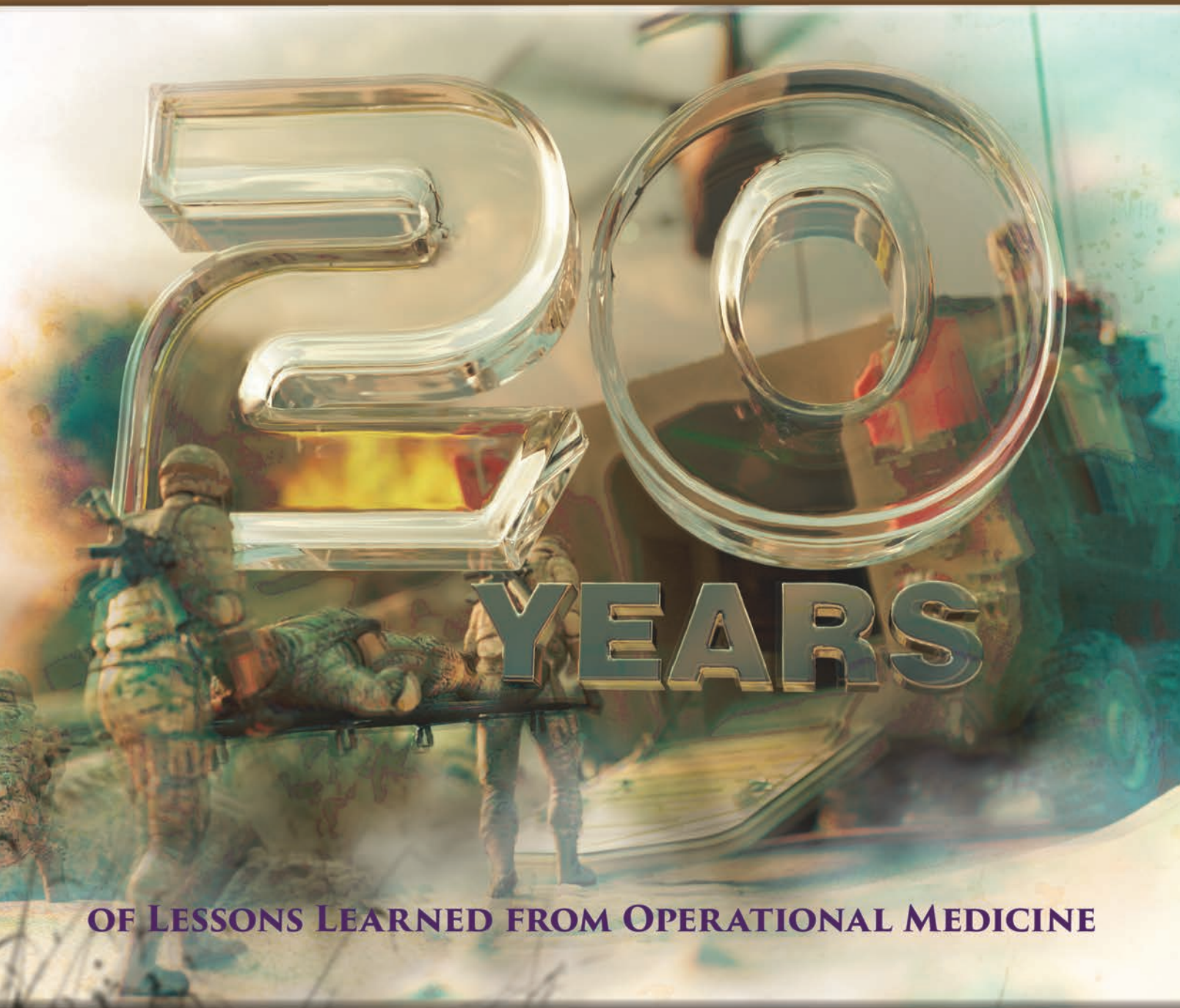
The Special Operations Medical Association's Official Journal

JSOM

JOURNAL *of* SPECIAL OPERATIONS MEDICINE™

Winter 2020
Volume 20, Edition 4

THE JOURNAL FOR OPERATIONAL MEDICINE AND TACTICAL CASUALTY CARE



OF LESSONS LEARNED FROM OPERATIONAL MEDICINE

A Peer Reviewed Journal That Brings Together The Global Interests of Special Operations' First Responders