# INTRODUCTION

In the United States, the incidence of sepsis was 4.1 to 81.4 per 1000 hospitalizations in 2016 - 2019. Moreover, the hospital mortality of sepsis was 6.5 - 32.9% from 2016 - 2019.[@10.1002/emp2.12782] These ratios were like the incidence and mortality of 2009 - 2014.[@10.1001/jama.2017.13836]. Over time, there have been little improvement in mortality.

In the case of sepsis, clinical standards are currently needed to better identify patients suspected of infection. Early diagnosis is particularly important because rapid management of sepsis patients has an important effect on improving outcomes[@]. According to the guideline[@Evans2021Surviving], intravenous antibiotic therapy should begin as soon as possible, ideally within one hour after sepsis, and early fluid resuscitation should start within the first three hours to stabilize sepsis-induced tissue hypoperfusion. Despite significant advances in our understanding of the pathologic mechanisms contributing to sepsis, no specific pharmacological therapy was available in sepsis. Therefore, still, the early diagnosis of sepsis is very important for the treatment of sepsis.

T cell have the important role in sepsis.

CD8+ T cells are also known as cytotoxic T lymphocytes (Tc cells) and play crucial roles in the eradication of intracellular pathogens and tumor cells[@He2021Immune]. T cell은 sepsis 첫 24시간 내에 INF-Ɣ, IL-17을 생성하여 innate immune function을 강화한다[@Kasten2010CELLS]. naive pathogen-specific CD8 T cell은 infection clearance와 memory CD8 T cell의 generation에 중요하다[@Danahy2016Clinicala].

Depending on the type of pathogen and pathogen biology, the peak number of Ag-specific effector CD8 T cells is achieved days to weeks after the initial infection. At this point, 95-98% of the expanded pool of Ag-specific CD8 T cells is eliminated during the programmed contraction (death) phase, with the surviving fraction encompassing a memory CD8 T-cell population with a protective capacity upon Ag re-encounter (re-infection) that depends on both the quantity and functional fitness of the CD8 T cell memory pool. These long-lived memory CD8 T cells undergo proliferative expansion upon pathogen re-encounter and provide increased protection after re-infection.

sepsis에서 T cell activation 이외에도 exhaustion 같은 현상이 생기면서 immune function이 감소한다.

Severe sepsis model에서는 activated T cell이 sepsis morbidity와 tissue injury를 증가시키고, less severe model에서는 functional T cell decrease mortality and bacterial loads를 보인다[@Kasten2010CELLS;]. - 예후를 보는데 cellular function이 중요하다.

The activation of T cells was characterized by a significant shift in DNA distribution towards a central DNA pattern and an increase in nuclear size.[@Gupta2012Developmental].

Although fluorescence (FL) is widely used as a standard method to visualize and analyze specific structures or molecules within a sample, there is increasing interest in exploring refractive index (RI) as an alternative method[@Jo2021Labelfree].

Despite the recognized need to quantify the levels of cellular immunity, the complexity and lack of scalability of these traditional methods (that is, ELISpot and flow cytometry), has so far prevented large-scale studies of the cellular immune response to COVID-19 in recovered and vaccinated individuals. Thus, most studies using ELISpot or flow cytometry assess only 10–40 participants, with larger clinical trials assessing around 200 (refs. 22–27). Furthermore, the process of freezing/thawing PBMCs, often used for testing T cell response, can introduce high variability in the results28,29, which can be bypassed by using whole blood.

# RESULTS

## Longitudinal [[3D RI Tomogram]] [[CD8+ T cell]] in sepsis patients

We acquired [[3D RI Tomogram]] on [[CD8+ T cell]]s from people with sepsis and controls to define the range of cell states present in these subjects, to identify differences in cell-state composition between groups, and to detect immune signatures that distinguish sepsis from the normal immune response to bacterial infection (Fig. 1) supplementary

## Different [[3D RI Tomogram]] distribution between septic shock status and healthy control

We found that the distribution of [[3D RI Tomogram]]s was significantly different between septic shock status and healthy control (Fig. 2).

status에 따른 mean median 차이가 있다.

## [[3D RI Tomogram]] correlates with clinical value in sepsis patients

## [[3D RI Tomogram]] distinguish varying sepsis status

## Visual explanation of the deep learning model

We used [[Grad-CAM algorithm]][@Selvaraju2020GradCAM] to identify image regions that contributed the most to the prediction made by deep learning. Representative examples of malignant colonoscopic images with accompanying saliency heatmaps highlighting features most influenced the model prediction was shown in Fig. 4 and supplementary Data #.

# DISCUSSION

RI ~ function of the cell T1에서 보이는 변화는 CD8+ T cell의 activation으로 인한 변화이지 않을까? T cell의 활성화가 central DNA pattern과 increase nuclear size로 대변된다[@Gupta2012Developmental].

기본적으로 cytokine release는 cell에서 일어나는데 이러한 cell의 변화가 먼저 생기고 cytokine release가 일어나는 것이다. 따라서 cell morphology를 측정하는 것이 더 빠르게 면역 반응을 볼 수 있는 방법이다.[@] Importantly, circulating substances might not represent the cellular response very well and other types of markers might be more relevant[@Cohen2015Sepsis].

Takes only 1 ml of blood and a 3-hour turnaround time. 다른 것들은 시간이 많이 걸린다.

In a single droplet of blood, there are many T cells present, and using only three or more images, we obtain almost 100% performance.

In conclusion, our approach represents a flexible strategy that can be easily used to detect the presence of T cells even help to diagnosis and predict prognosis of sepsis.

# REFERENCE

# MATERIALS AND METHODS

## Study samples and clinical adjudication

The septic shock cohorts comprised subjects with septic shock who presented to the ED at the Severance hospital in Korea. Informed consent was obtained from subjects or their surrogates. To define the trajectory of septic shock, for each individual, data were collected and blood samples were drawn with EDTA Vacutainer tubes (BD Biosciences) near septic shock diagnosed ([[T1]]), septic shock was resolved ([[T2]]), and before discharge ([[T3]]). The study was approved by the institutional review board of Severance Hospital, Yonsei University Health System, Seoul, Korea, and all patients provided informed consent (IRB No.4-2021-1236).

The study cohort was enrolled in the ED at the Severance Hospital from April 2022 to June 2022. They consisted of people with septic shock, defined by Sepsis-3 consensus definitions[@], specifically, (1) with suspected infection, (2) 2 or more criteria of qSOFA, which consists of respiratory rate >= 22/min, altered mentation, and systolic blood pressure <= 100 mmHg, were satisfied, (3) an acute change in total SOFA score[@] ≧ 2 points consequent to the infection. Study samples were collected within 12 h of subject’s arrival at the ED. Patients with followings were excluded: (1) age under 19, (2) pregnant or lactating, (3) active cancer status (4) acute stroke, (5) acute cardiovascular disease, (6) acute burn, (7) acute gastrointestinal bleeding or within the last three months of bleeding, (8) taking immunosuppressive drugs after organ transplantation, (9) taking immunosuppressive drugs for autoimmune disease, (10) immunodeficiency conditions that have been diagnosed before, or that have CD4 cell counts below 350 G/L, (11) neutropenia (neutropenia < 500 G/L) or if the neutrophils were 500-1000 G/L due to chemotherapy and were expected to decrease, (12) diagnosed with adrenal dysfunction, (13) prescribed steroid equivalent to or greater than 0.5 mg/kg/day prednisone, (14) active tuberculosis, (15) cystic fibrosis, (16) post-traumatic, (17) who needed immediate surgery, and (18) the state of Do-Not-Resuscitate (DNR). The study was approved by the institutional review board of Severance Hospital, Yonsei University Health System, Seoul, Korea, and all patients provided informed consent.

The healthy cohorts consisted of a total of 20 volunteer healthy patients as a control group to compare with a cohort of septic shock patients.

## Isolation PBMCs from whole blood

Peripheral whole blood was collected in EDTA tubes and processed fresh via Ficoll-Paque Plus separation (GE Healthcare, 17144002). The blood was first diluted with 5 ml 2 mM EDTA-PBS (Invitrogen, 1555785-038), before 10-20 ml of diluted blood was carefully layered onto 15 ml of Ficoll in a 50 ml falcon tube. The sample was centrifuged at 900g for 30 min at room temperature. The plasma layer was carefully separated and the PBMC layer was collected using a sterile Pasteur pipette. The PBMC layer was washed with three volumes of EDTA-PBS by centrifugation at 500g for 5 min. The pellet was suspended in EDTA-PBS and centrifuged again at 400g for 5 min. The PBMC pellet was collected, and the cell number and viability were assessed using Trypan blue (The Countess II Automated Cell Counter, ThermoFisher, USA).

## Sorting cells

The magnetic activated cell sorting (MACS), a typical method for isolating the cells from a mixed population, was used for cell sorting (MACS from Miltenyi Biotec, Bergisch Gladbach, Germany)[@]. The [[CD8+ T cell]]s were negatively selected by MACS. Isolated cells were kept at 4°C to keep the viability of the cells. 80 μL isolation buffer and 20 μL MicroBeads were added and mixed the cells in the incubator for 15 minutes at a temperature 4°C. By setting the magnetic stand and column for sorting we first equilibrate the column by washing 3mL of the isolation buffer. Next to collect each of lymphocytes, we withdrew the column from the magnetic rack and set up a conical tube. By adding 5mL of isolation buffer, we pumped the solution through the column to extract the final sorted cells from the collection tube.

## 3D RI tomography setup and data acquisition

We took three-dimensional (3D) refractive index (RI) images of the CD8 T cells with 3-D quantitative phase imaging (QPI) system [@], which is commercialized and dubbed holotomography (HT-2H, Tomocube Inc., Daejeon, Republic of Korea) [@]. The digital micromirror device scans the various illumination angles and the 3D RI tomogram is reconstructed from the sinogram of 2D QPI measurements under the principle of optical diffraction tomography [@].

## RI distribution 보는 방법 (shell)

## Image preprocessing and data augmentation. (종현 - 아래 주석 참고해서 다음 내용 수정해주세요)

## CNN training (종현 - 아래 주석 참고해서 다음 내용 수정해주세요)

## Visual explanation

We used Grad-CAM[@] to derive visual explanation by localizing the image area that most influences the decision made by the deep learnig model.

## Statistical analysis

We used PR curve and ROC curve to describe the classification ability of [[the deep learning model]]. The PRC was demonstrated to be more informative than receiver-operating curve on imbalanced datasets[@]. We created the PR curve by plotting recall rate (also known as sensitivity) against precision (also known as positive predictive rate) by varying the predicted probability threshold. The ROC curve was created by plotting recall rate against negative predictive rate (also known as specificity). The F1 metric is defined as harmonic mean between precision and recall rate, which is calculated as F1 = 2 \_ precision \_ recall/(precision + recall). The 95% confidence intervals for sensitivity, specificity, positive predictive rate, and negative predicted rate were calculated by the Clopper-Pearson method[@]. We plotted PR curve and calculated the AUPRC with R package PRROC[@] (version 1.3.1). We plotted the ROC curve and AUROC with R package pROC (version 1.10.0)[@]. Statistical analysis was conducted with R software (version 3.4.3)[@]. We used AUPRC as the primary outcome to measure the performance of [[the deep learning model]]. We used precision, recall rate and F1 score when comparing the classification ability of CRCNet with endoscopists.

## Reporting Summary

Further information on research design is available in the Nature Research Reporting Summary linked to this article.

# Data availability

# Code availability

# Reference

# Acknowledgements

# Competing interests

# Additional information