DIAGNOSTIC DEVICES

Detecting sepsis by observing neutrophil motility

A microfluidic device for assaying neutrophil motility in blood samples from sepsis patients and a machine-learning algorithm trained with the motility data enable a faster and accurate sepsis diagnosis.

Umer Hassan, Enrique Valera and Rashid Bashir

Sepsis — a complex immune-system disorder initiated by an infection or an insult to the immune system — strikes more than 1 million people per year in the United States, at an overall cost of about US$24 billion to the US healthcare system[1]. Approximately 230,000 of these patients die, a number that is greater than the number of deaths in the US from prostate cancer, breast cancer and AIDS combined[2]. Also, on the basis of clinical data, the incidence of sepsis and the related mortality have remained the same between 2009 and 2014[3]. A dominant factor underlying these numbers is the rapid progression of the condition and the lack of an accurate and rapid sepsis-stratification method. Microbial-culture methods for sepsis diagnosis currently take up to 6 days, and have a high false-negative rate[4]. For sepsis patients, every hour of delay of the start of an appropriate antimicrobial medication leads to a roughly 7.6% decrease in 72-hour survival rates, and patients who survive have a continuing risk of mortality after discharge[5]. This highlights the urgent need for techniques for early diagnosis[6].

Clinical scores based on the assessment of sequential organ failure are recommended in current clinical practice, but they are largely non-specific and lead to high rates of misdiagnosis and to poor prognoses[7,8]. Although some molecular and cellular biomarkers correlate with specific sepsis stages, new biomarkers that combine with data from electronic medical records[9] to provide physicians with information on the state of sepsis progression, the state of the immune system and, most importantly, a precise diagnostic method, are urgently needed. Reporting in *Nature Biomedical Engineering*, Daniel Irimia and colleagues now show that the spontaneous motility of neutrophils in a drop of blood can be used to diagnose sepsis faster and with higher accuracy[10] (over 98% across two independent cohorts of 42 patients) than available methods. To aid the diagnosis, the researchers developed a machine-learning algorithm to identify the most relevant neutrophil-motility parameters (related to the migration distance and number of migrating neutrophils in the microfluidic channels) and combined them into a 'sepsis score' that differentiated patients with sepsis from those without. They validated the sepsis score in a double-blinded, prospective case-control study, for which diagnoses were over 95% specific and sensitive.

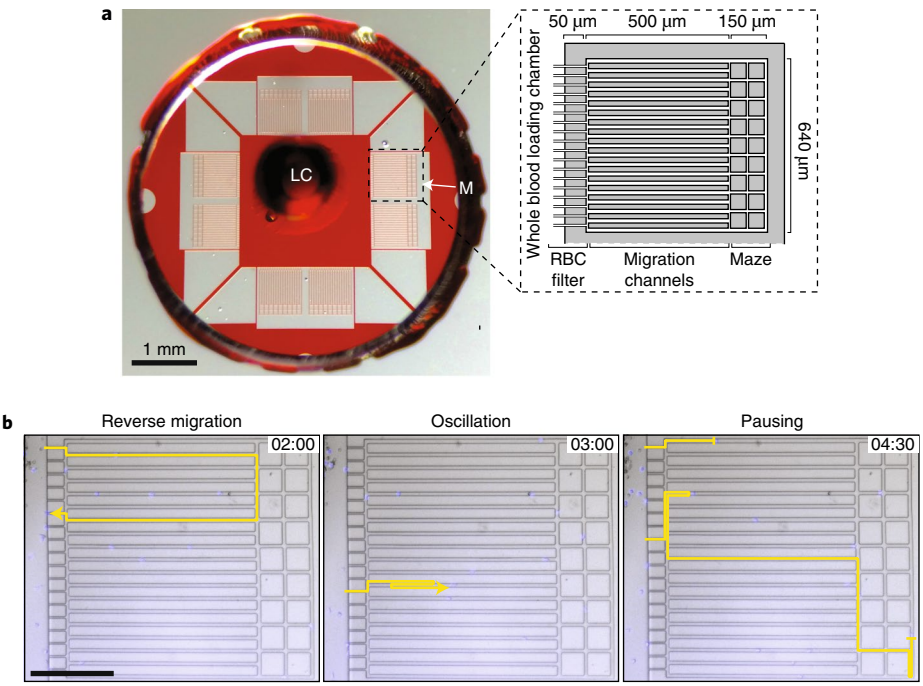

Fig. 1 | Microfluidic device for assaying the spontaneous motility of neutrophils in whole blood. **a**, Microfluidic chip, consisting of a central loading chamber (LC) and four mazes (M; inset) of microfluidic channels. RBC, red blood cell. **b**, Three migration patterns of neutrophils from a sepsis sample in a maze of microfluidic channels. The numbers in the top right corner are the time stamps, in hours:minutes, of the corresponding frames in a time-lapse video of the migration patterns. Scale bar, 100 µm. Figure adapted from ref. [10], Macmillan Publishers Ltd.

Irimia and co-authors' microfluidic chip features a central region for placing a drop of diluted whole blood (Fig. 1a). Blood cells then migrate into a maze of etched channels. A filter placed before the inlets of the channels allows the neutrophils, but not red blood cells or leukocytes, to enter the channels. The spontaneous motility of the neutrophils is optically recorded for about 4 hours and then analysed to quantify their motility and migration. A supervised machine-learning algorithm narrowed down 13 motility parameters to a set of 5 (neutrophil count, number of oscillations within a channel, pause time between movements, reverse migration out of the channels, and average distance traversed by the neutrophils; Fig. 1b) that resulted in the highest prediction accuracy. The authors used these 5 parameters to build the sepsis score.

Neutrophils are known to play a critical role in the innate immune system, because they circulate in the bloodstream and are the first cells to migrate to sites of infection and fight the infection source[11] (typically, foreign microbes). Irimia and co-authors

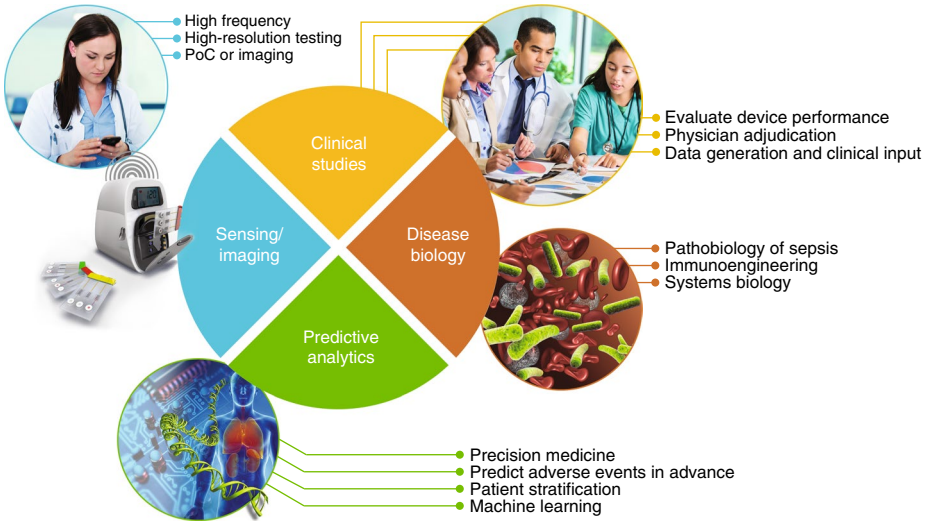

Fig. 2 | An integrative approach for the diagnosis and stratification of sepsis. The biology of sepsis, clinical testing, predictive analytics and machine-learning approaches, and new point-of-care (PoC) sensors and devices, are all needed for the development of precise and accurate diagnostic tools with clinical utility. Figure created by Janet Sinn-Hanlon, The DesignGroup@VetMed, University of Illinois at Urbana-Champaign. Image credits: top-left (blue circle), gpointstudio / iStock / Getty Images Plus; top right (yellow circle), Steve Debenport / E+ / Getty Images Plus; bottom left (background in green circle), archy13 / iStock / Getty Images Plus.

demonstrate that the spontaneous motility and migration of neutrophils is linked to patient state rather than to other factors, such as the activation of the cells due to post-processing. To test this, they compared the motility of the cells from diluted whole blood with that of purified neutrophils isolated from the same patient blood samples. Only for the diluted whole-blood samples were all 5 motility parameters higher for the samples from patients with sepsis (for the isolated-neutrophil samples, reverse migration and average migration distance were negatively correlated with the presence of sepsis). Also, to eliminate effects from extracellular and cell-autonomous signalling factors, the authors exchanged plasma and neutrophils between blood samples from septic and healthy donors. Interestingly, they found that the neutrophils from septic patients exhibited high spontaneous motility even in healthy plasma, which indicates that activated neutrophils remain so for some time. Moreover, neutrophils from healthy subjects exposed to plasma from septic patients exhibited high spontaneous motility, indicating that the signals from the plasma of septic patients can activate normal neutrophils. Yet spiking whole blood with different individual immune-modulators known to be elevated in septic blood did not induce the highly motile neutrophil phenotype.

The effective clinical diagnosis and stratification of sepsis will require a multidisciplinary approach that encompasses the underpinnings of sepsis pathobiology, clinical studies with patient samples and endpoint adjudication, the quantification of sepsis biomarkers, and data analytics and machine learning (Fig. 2). Irimia and collaborators' study brings insights from all these areas. Although many biomarkers (proteins and cytokines such as procalcitonin, interleukin-6 and C-reactive protein, nucleic acid molecules such as miRNA, cell-surface-receptor expression such as CD64 on neutrophils[12] and CD11b and HLA-DR on monocytes, and the mechanical stiffness of neutrophils[13]) have been shown to correlate with sepsis, because of the complexity of the syndrome, it is generally accepted that no single biomarker will be highly predictive of sepsis. The motile phenotype of neutrophils in Irimia and colleagues' assay results from the 'integration' of signals from a variety of biomarkers. In fact, neutrophils exposed to a set of specific cytokines, including interferon-γ (IFN-γ), tumour-necrosis factor-α (TNF-α), granulocyte-colony stimulating factor (G-CSF), interleukin-1 β (IL1-β) and lipopolysaccharides, did not produce the motility phenotype, which suggests that in the authors' assay neutrophils experience a complex interplay between a larger number of parameters. Should a correlation between motility parameters and neutrophil stiffness exist, high-throughput methods for the measurement of neutrophil stiffness could also make powerful diagnostic assays. Also, because neutrophils in vivo move to the site of infection via chemotaxis, setting up artificial chemical gradients in a microfluidic chip could provide additional insights or alternative methods to more quickly assess cell motility and migration.

Irimia and colleagues' microfluidic assay provides a sepsis score in about 6 hours (4 hours of microscopy observation and 2 hours of data analysis), yet building a larger training database for the machine-learning algorithm could reduce the observation time. And automated electrical means to measure the movement of the neutrophils should reduce the instrument size and improve its portability. Although validation through larger clinical trials is warranted, neutrophil motility has significant clinical potential as a highly prognostic biomarker. ❐

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