Videocapillaroscopy of the oral mucosa as an non-invasive tool for early detection of sepsis

Gabriel Petre Gorecki[1,2], Cosmin Moldovan[3,4], Elena Rusu[3], Daniel Cochior[3,5,6]

[1] Doctoral School of Medicine, IOSUD Titu Maiorescu University of Bucharest, Romania
[2] Bucur Maternity Hospital, "Sf. Ioan" Emergency Hospital, Bucharest, Romania
[3] Faculty of Medicine, Titu Maiorescu University of Bucharest, Romania
[4] General Surgery Clinic, CF1 Witting Clinical Hospital, Bucharest, Romania
[5] General Surgery Clinic, Monza Clinical Hospital, Bucharest, Romania
[6] Sanador Clinical Hospital, Bucharest, Romania

ABSTRACT

Introduction. Due to the high incidence, morbidity, mortality and high costs for the health system, sepsis stands out, among other things, by the firm indication of hospitalization in an intensive care unit. Dysfunction of microcirculation plays a central role in the genesis and maintenance of septic syndrome, as it represents a pathophysiological milestone of this syndrome. Videocapillaroscopy opens up the possibility of detecting microvascular anomalies in the early stages, allowing for new diagnostic and research opportunities.

Material and method. Our unrandomized, prospective, multicenter, analytical study analysed a group of 51 patients with various ethology of septic shock, a clinical condition that was diagnosed on the basis of standard clinical and biological criteria, hospitalized in three ICU clinical wards, over the course of 15 months (August 2019 - November 2020), where the parameters of the oral mucosa microcirculation were monitored with the help of a videocapillaroscope, the resulting prototype of an invention patent already implemented by the authors.

Objectives. The main objective of our study was to validate, on a clinical level, the possibility of using videocapillaroscopy of the oral mucosa, as an efficient means of early detection of the changes that predict the early onset of septic shock in patients in ICU patients.

Results. Videocapillaroscopy was performed with a prototype device, recording 357 images of the microcirculation of the oral mucosa in the patients that had already been confirmed, via classical clinical and paraclinical means, with the diagnosis of sepsis. Discussions. The statistical analysis of the essential 5 elements detected by videocapillaroscopy (capillary density, incidence in normal and parallel disposition, diameter and microhemorrhage) corroborated with the clinical and paraclinical elements of the sepsis state path clearly highlights the correlation power of these determinations.

Conclusions. Given the completely non-invasive nature of this imaging method, the very low costs associated with the method, the ease in collecting and interpreting data as well as the results that bear strong statistical correlation, we can conclude that it can successfully be considered a method of prediction with high reliability and specificity in the early detection of sepsis, regardless of its etiology.

Keywords: sepsis, septic shock, early detection, videocapillaroscope, mucosa, oral

INTRODUCTION

Sepsis is a life-threatening dysfunction of organs caused by an inappropriate response of the host to infection [1]. Sepsis and septic shock are major health problems, impacting millions of people around the world each year, killing between one in three and one in six of those it affects [2-4]. Early identification and proper management in the first hours after the development of sepsis significantly improves the results [5]. Signs and symptoms of sep-

sis are nonspecific and often mimic other ailments [6-9]. Since there is no „gold standard" test to diagnose sepsis, its differential diagnosis in a patient with organ dysfunction is very difficult. Statistically, a third or more of the patients initially diagnosed with sepsis turn out to have non-infectious conditions [6][10].

Therefore, microcirculatory dysfunction is a key element in the pathophysiology of sepsis. Videocapillaroscopy is a fundamental non-invasive technique allowing the visualization of peripheral circulation and the study of microangiopathy, which occur in numerous pathologies, both in the diagnostic and monitoring phases of the disease. Using this technique, any disease that affects the microcirculation can be monitored (in a timely and non-invasive manner), making it a method of high interest in the study of blood microcirculation, since it facilitates the examination of small vessels in patients, in vivo. The alteration of the capillary microcirculation in a certain sector can be, in fact, the only definite documentation of the onset of a disease.

The literature on capillaroscopy has documented how data on position, shape, capillary caliber, and architectural framework can vary, depending on the center it was collected from [11]. To our knowledge, a simple, non-invasive method, such as videocapillaroscopy, has never been used in our country to observe the characteristics of microcirculation of the human oral mucosa in sepsis. The morphological study of microcirculation is of fundamental importance, mainly because the microvascular bed is directly involved in the etiopathogenesis of sepsis. The value of capillary investigation as a means of diagnosis in cases of peripheral microcirculation damage has been confirmed by numerous studies. Using this method, Haljed showed that diabetic patients have abnormalities in the regulation of capillary flow [12]. Other studies have used capillaryoscopic investigations to assess the deterioration of microcirculation not as a complication of the disease (diabetes), but as an initial stage, and therefore placing the diagnosis. The definition of normal or pathological conditions can be dictated by differences in the diameter or length of the capillary loop, architectural disorders or the presence of morphological abnormalities and micro-hemorrhages. Existing morphological data seem highly relevant, as they would certainly be altered during specific systemic pathologies (systemic sclerosis, autoimmune pathologies, potentially malignant lesions, malignant lesions, inflammatory diseases, sepsis) and even in the case of exposure to certain risk factors, such as smoking [11]. Consequently, videocapillaroscopy is an interesting way to study microcirculation due to the possibility of examining small vessels in vivo by means of a dedicated device.

MATERIAL AND METHOD

Objectives

As a main objective, our study tried to clinically validate the possibility of using videocapillaroscopy of the oral mucosa, as an effective means of early detection of changes that predict the onset of septic shock in patients in the ICU. On a secondary level, the study also tried to identify the threshold values of the elements provided by the capillaroscopy that would allow the support of the early diagnosis of sepsis.

Description of the cohort under consideration

This study is of a prospective, controlled, analytical, non-abdominal, non-control group, multicenter type, in which 51 patients were enrolled.

The group consisted of 51 patients, aged between 18 and 91 years, hospitalized with the diagnosis of sepsis/septic shock, of various etiologies, in the 3 ICU clinics belonging to the 3 hospitals where the doctoral study was carried out, during July 2019 and November 2020.

The multicenter component was ensured by involving the 3 ICU clinics that are part of the following hospital units: Targoviste County Emergency Hospital – SJUT coded in the database; Ilfov County Hospital – ILF coded in the database; Giurgiu County Emergency Hospital – coded SGR in the database.

The criteria of inclusion in the study consisted of patients admitted, either directly or by inter-hospital transfer or from their own wards, in the ICU for suspicion of severe septic sepsis/shock, regardless of etiology, regardless of age or comorbidities and who gave their informed consent. The exclusion criteria consisted of patients who did not give their informed consent, those in whom the suspicion of sepsis was not claimed, i.e. those in advanced or terminal phases of the evolution of a severe septic sepsis/shock in which consent could not be obtained, and the patients where obtaining the oral mucosae parameters and characteristics would not have been feasible.

The elements recorded and highlighted for analysis in the study considered both the clinical component of the diagnosis and the evolution of the septic sepsis/shock state, but especially the parameters obtained by non-invasive video-capillaroscopy of the oral mucosa. All these elements were listed in the database of the study, on which specific statistical processing and analysis were carried out.

Analyzed elements and the method of obtaining the parameters

The data set recorded by the study was divided into three distinct categories: **general and passport**
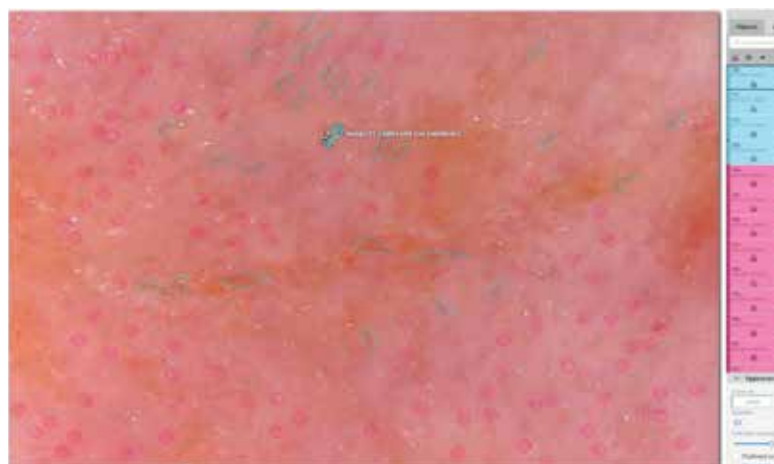
FIGURE 1. The process of manual marking, by polygons, of the capillaries detected in the analyzed frame. In this example, capillaries in parallel incidence were identified on the plane of the videocapillaroscope camera.

data (such as sex, age, number of images taken, date of admission, discharge, respectively the number of days in the ICU), **clinical elements** (associated pathology, type of biological sample from which the cultures were taken, types of germs identified by cultures, antibiogram, the number of leukocytes, platelets, the level of hemoglobin, lactate and C-reactive protein as well as the temperature) as well as **elements determinable by videocapillaroscopy** (number of capillary loops in incidence parallel to the mucosa, in perpendicular incidence, the density of capillaries, their size and the presence or absence of micro-hemorrhages).

Working methodology

The following steps were taken:

Examination of the patient and acquisition of images. The examination was made after calibrating the videocapillaroscope by balancing the white point, observing the rules of asepsis and antisepsis, through which a video of a predetermined length of 60 seconds was recorded.

Preparation of images. The images stored on the capillaroscope memory card were manually analyzed to identify some of the characteristics pursued by the study, later being uploaded to the online software platform CVAT (Computer Vision Annotation Tool - https://cvat.org) where polygons were used to manually identify these elements. Based on these markings, the software was able to automatically determine the number of capillaries (Figure 1).

Statistical analysis

The statistical analysis was based on a Microsoft Excel 365 spreadsheet document through which the primary statistical processing was performed and the advanced processing (Wilcoxon-Mann-Whitney© test, ANOVA unisens test as well as the exact Fisher test) was performed with the IBM SPSS© software ver. 24 but also with the CDC Epi Info© ver

7.2.2.1. To evaluate the accuracy of statistical processing, the P value was set to 0.05.

The study received the approval of the research ethics committee from the Doctoral School of Medicine within IOSUD Titu Maiorescu University of Bucharest, based on the research ethics approvals obtained in advance from each of the hospital units participating in the study. The agreements shall be available on request.

RESULTS

The analysis of the demographic data of the 51 patients in the analyzed group, coming from all the clinics participating in the doctoral study, revealed the following characteristics: a higher male patient component (19 women and 32 men respectively), with a B/F ratio of 1.68:1; the average age to be recorded was 67 years with extremes ranging from 18 to 91 years.

The analysis of the case participation of the 3 clinics where the study revealed that the main share is held by the clinic with the SJUT identifier, with 37 cases (72.5%) followed by the ILF, with 9 cases (17.6%) and SGR with 5 cases (9.8%).

The analysis of the comorbidities presents in the participating patients that are significant for the analyzed pathology reveal a preponderance of those of cardiovascular nature (33 cases, 65% of the group), followed in descending order by the hepatic ones (14 cases, 27%), COPD (13 cases, 25%), obesity and CKD with equal weights (11 cases, 22%) respectively, with the lowest prevalence, type 2 DM with 4 cases (8%).

The number of days of hospitalization in the ICU of patients with sepsis, reveals an average number of 12 days, with extremes from 1 day to 59 days.

Regarding the evolution towards recovery or exitus of patients with sepsis and septic shock participating in the study, a marginal preponderance of those with exitus compared to those who survived

and could be transferred outside the ICU services in those hospitals was shown. Thus, a survival rate of 47% is recorded, with a mortality of 53%.

Out of the total of 51 patients admitted to the ICU wards, twelve (a share of 24% of the total group) had the diagnosis of sepsis placed based on the various biological product cultures taken. The analysis of the distribution of biological samples, showed that, the most frequently used biological products were the tracheobronchial aspirate (8 situations, 16% of the total samples, 47% of the patients whose collections were sampled), followed by uroculture (4 situations, 8% of the total samples, 24% of the collections) followed by hemocultures with 2 situations (4% of the total samples, 12% of the collections). In the category „other types of samples" we have classified special situations represented by: sputum and inguinal swab, with a share of 3% of the total samples, 6% of the collections.

The analysis of the germ types found in the cultures performed on the described samples reveals the preponderance, in an equal manner, of *Staphylococcus aureus* (MRSA) and *Acinetobacter baumannii*, with 4 cases each, followed by 2 cases of *Pseudomonas aeruginosa* with 2 cases respectively *Staphylococcus simulans, Acinetobacter lwoffii, Enterococcus, Escherichia coli, Candida albicans, Candida glabrata* with 1 case each.

The combined analysis on several levels, of which the primary level is the status at discharge (exitus or transfer outside the ICU, equating to the removal of the patient from the state of sepsis), the secondary level represented by the sex, followed by the tertiary level of analysis being the age, with the individual calculation of the number of days spent in the ICU reveals that, from the category of the deceased, the maximum peak is reached by men of 54 years (55 days ICU), being followed by those of 84 years (44 days in the ICU), unlike women whose peak was reached in the case of those with the age of 70, with 40 days in the ICU. From the category of

those that recovered, in the case of men, the maximum number of days of ICU is lower, with 59 days for the age of 45, whereas, in the women population, the age of 68 years being the category where most days of ICU hospitalization were recorded, more precisely 41 days.

Analyzing all the biological and clinical elements in the studied group, in both states, septic and non-septic respectively (Table 1) the evolution of leukocytes (LEU) recorded an increase of 259% for the minimum values and 195% for the maximum values, respectively, between the two clinical situations. For hemoglobin (HLB), the analysis determined a variation of 87% (for the minimum values) and 93% for the maximum values, between the sepsis situation and non-sepsis respectively. The evolution of platelets (PLT), compared between clinical situations of sepsis and non-sepsis, led to an increase of 122% (for the minimum values) and of 132% for the maximums, between the two clinical situations. Also, the evolution of lactate (LACTATE) led to an increase of 140% (for the minimum values) and of 117% for the maximum values, between the two clinical situations. The evolution of PCR values determined an increase of 2067% (for the minimum values) and of 167% for the maximum values, between the two clinical situations.

According to the design of the study, videocapillaroscopic determinations were performed coupled to the sets of analyzes performed at key moments of the clinical evolution of patients. Thus, each patient benefited from 5 complete biological determinations, each such set being accompanied by a videocapillaroscopic investigation, totaling a total of 255 determinations that generated 355 files with significant images for the analysis of the relevant parameters.

Analyzing, comparatively, the totality of the elements determined by videocapillaroscopy, both in the situation of sepsis and non-sepsis (Table 2), it can be seen that, between non-septic and septic situ-

TABLE 1. Comparative evolution of the averages of biological parameters in the study, in septic and non-septic states respectively

Param.	LEU						HGB					
State	Min	%	Max	%	Med	%	Min	%	Max	%	Med	%
non-SEPSIS	5,100		19,000		11,350		6.70		10.70		9	
SEPSIS	13,200	259	37,130	195	21,000	185	6	87	10	93	9	91
Param	PLT						LACTATE					
State	Min	%	Max	%	Med	%	Min	%	Max	%	Med	%
non-SEPSIS	145,000		365,000		236,500		0.86		2.90		1	
SEPSIS	177,000	122	483,000	132	310,000	131	1.20	140	3.40	117	2	115
Param	PCR											
State	Min		Max		Med	%						
non-SEPSIS	7.11		150.00		111							
SEPSIS	146.99	2067	250.34	167	147	133						

TABLE 2. Comparative evolution of elements determined by videocapillaroscopy of the oral mucosa in patients with sepsis states, respectively, non-sepsis

| Param.	Cap_Vector-A						Cap_Vector-B					
State	Min	%	Max	%	Med	%	Min	%	Max	%	Med	%
non-SEPSIS	81		101		91		39		66		52.5	
SEPSIS	150	185	171	169	160.5	176	120	307	151	228	135.5	258
Param	Cap_Total						Cap_Desity					
State	Min	%	Max	%	Med	%	Min	%	Max	%	Med	%
non-SEPSIS	120		167		143.5		44		63		53.5	
SEPSIS	270	225	322	192	296	206	92	209	110	174	101	188
Param	Cap_Calibre						Cap_Microhem					
State	Min		Max		Med	%	Min		Max			
non-SEPSIS	9		15		12		0		0			
SEPSIS	19	211	63	420	41	341	1		1			

ations, all parameters register significant percentage increases, from 176% for capillaries in parallel incidence (Cap_Vector-A) to 258% for capillaries in incidence perpendicular to the mucosal (Cap_Vector-B); also their density increases, on average, by 188%, as well as the average diameter, by 341% (Cap_Density). The only parameter that evolved into absolute values was Cap_Microhem, responsible for monitoring microhemorrhage, and which was present only in the states of sepsis and absent in non-septic ones.

In order to be able to determine the degree of correlation of the elements determinable by video-

FIGURE 2. Comparative analysis, between septic or non-septic states, of biological data in patients in the studied group

FIGURE 3. Comparative analysis, between septic and non-septic states, of the data obtained by videocapillaroscopy of the oral mucosa in the patients in the studied group

capillaroscopy with the clinical ones, a direct comparative analysis was performed, from which it is possible to observe the profile of the characteristics that define the relatively normal aspect of the oral mucosa in these patients, preceding the states of sepsis or septic shock, compared to the sepsis situations (Figure 2 and Figure 3).

The application of the statistical analysis on the data obtained (Pearson and ANOVA test) that shows the evolution of the minimum, maximum and average values of the biological parameters, as well as from the comparative analysis, between the two clinical situations, of sepsis and non-sepsis, of the two data sets, add up to the general clinical picture of the patient with sepsis, within our study, which serves as a template for the confirmation of the videocapillaroscopic data. Therefore, it can be noted that an average increase of 185% of the LEU is suggestive of the state of sepsis ($p<0.05$, CI: 97.4), a 130% increase in PLT is suggestive of the state of sepsis ($p<0.05$, CI: 96.3), a 115% increase in LACTATE is suggestive of the state of sepsis ($p<0.05$, CI: 97.2) respectively a 133% increase in PCR is suggestive of the state of sepsis ($p<0.05$, CI: 98.2). However, the HGB analysis does not reveal a statistically significant change between the two clinical situations of sepsis and non-sepsis ($p>0.9$, CI: 34); therefore, monitoring this parameter does not prove useful to the study.

The analysis of Table 2 shows that there is a relatively constant ratio between the two types of capillaries, normal and parallel in incidence on the mucosa, in favor of parallel ones, in the case of a mucous membrane corresponding to a non-septic status of the patient. If we analyze the parameter that tracks the presence/absence of microhemorrhage in the oral mucosa (Cap_Microhem), we will see that the positivity of the determination is recorded only in sepsis situations. The association of microhemorrhages with the biological elements of sepsis has a strong statistical significance, with a correlation of $p=0.004$ and a CI of 99.4%. Basically,
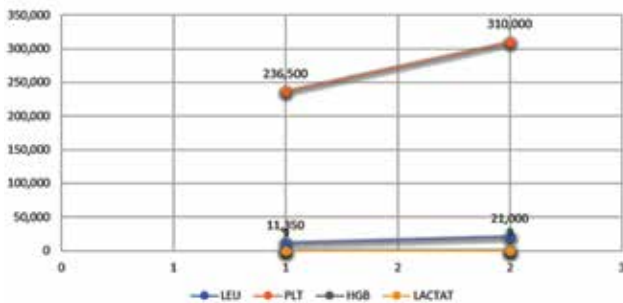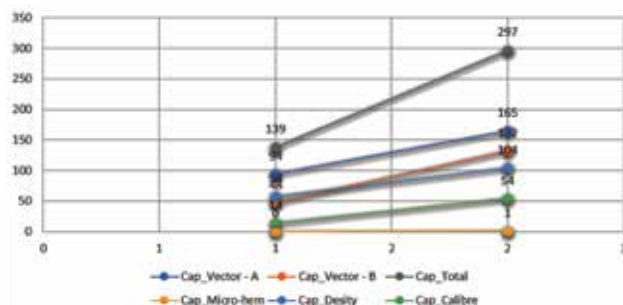
we can therefore say that the identification of these microhemorrhages in a patient with clinical sepsis criteria is a videocapillaroscopic element of certainty. Analyzing the increase in the density of capillaries, compared to clinical situations with and without sepsis, corroborated with biological data (Figure 2 and Figure 3), we will notice that there is correlation with important statistical significance (p=0.05, CI: 96.4%). The analysis of the distribution of capillary diameters in clinical situations of sepsis respectively non-sepsis as well as the comparative analysis of this parameter between the two situations reveals a significant statistical correlation (p=0.04, CI: 97.3).

DISCUSSIONS

Although capillary studies have been carried out and published in the literature [13-19], they have fully targeted only determinations made from the nail capillary bed and for chronic pathologies, such as lupus erythematosus, rheumatoid arthritis, diabetes mellitus, pathologies that allow monitoring over longer periods. Videocapillaroscopic studies that analyze and parameterize only the appearance of oral microcirculation are also very few in number [20,21], and do not treat aspects of correlation with systemic pathology with acute potential, such as sepsis, being also limited to chronic pathology, such as Behcet's syndrome. Being a first, the results of our study should be interpreted as such but, given the known limitations, they certainly require the expansion of research to validate routine use in medical practice in ICU services.

The changes in the biological determinations in the patients with sepsis in the study are similar to those published in the recent literature [9][22][23], with all parameters recording congruent variations, with the exception of hemoglobin, which, in our study, showed a plateau evolution, as opposed to larger studies, in which there was an amplitude variation of 5% [24].

Due to the limitations of the study, the investigation of the refill rate of the oral microcirculation capillaries was not possible, this determination requiring equipment and software solutions that would not have made it possible to follow the patient with the same dynamics as the rest of the parameters, so it is difficult to refer to studies such as that of Hernandez et al [25], a study that only focuses on the evolution of this parameter in septic shock.

Known limitations of the present study

Although the study has clinical and statistical validation, it has certain limitations, namely **the relatively small number of videocapillaroscopic pa-rameters actually analyzed**, namely only 5 of the total ones that can be effectively quantified and analyzed [16-18][26-28], **the relatively small number of patients with a definite diagnosis of sepsis and septic shock** of only 51 patients, although with statistical significance, it is, however, a relatively small population to draw universal conclusions about the possibility of using videocapillaroscopy in all possible etiologies involved in the diagnosis of sepsis and severe septic shock. In addition, **the lack of a control group for the study of oral microcirculation morphology** may constitute a limitation due to the absence of a basis for comparison.

CONCLUSIONS

All the parameters determined by videocapillaroscopy had significant statistical correlations with the biological parameters necessary to establish the diagnosis of sepsis, also their variations, at the transition from the non-septic state to the septic one, had a correlation with important statistical significance, thus representing a very valuable non-invasive research and diagnostic method in highlighting the peripheral microcirculation and in the study of the microangiopathy that frequently accompany the clinical manifestations from sepsis.

Given the completely non-invasive nature of this imaging method, the very low costs associated with the method, the ease in collecting and interpreting data, as well as the results with strong statistical correlation, we can assume that it can successfully represent a prediction method with high reliability and specificity in the early detection of sepsis, regardless of etiology. Of course, the relatively limited series of only 51 cases represents only a starting point for the subsequent definitive validation of the method in order to be able to issue recommendations for the actual routine use in the medical practice of ICU services.

Conflict of interest
The authors certificate that they have no financial or personal relationships that might bias the content of this work.

REFERENCES

1. Singer M, Deutschman CS, Seymour CW et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). *Jama.* 2016 Feb 23;315(8):801-10.

2. Fleischmann C, Scherag A, Adhikari NK et al. Assessment of Global Incidence and Mortality of Hospital-treated Sepsis. Current Estimates and Limitations. *American journal of respiratory and critical care medicine.* 2016 Feb 1;193(3):259-72.

3. Fleischmann-Struzek C, Mellhammar L, Rose N et al. Incidence and mortality of hospital- and ICU-treated sepsis: results from an updated and expanded systematic review and meta-analysis. *Intensive care medicine.* 2020 Aug;46(8):1552-62

4. Rhee C, Dantes R, Epstein L et al. Incidence and Trends of Sepsis in US Hospitals Using Clinical vs Claims Data, 2009-2014. *Jama.* 2017;318(13):1241-9.

5. Paoli CJ, Reynolds MA, Sinha M et al. Epidemiology and Costs of Sepsis in the United States-An Analysis Based on Timing of Diagnosis and Severity Level. *Critical that medicine.* 2018 Dec;46(12):1889-97.

6. Klein Klouwenberg PM, Cremer OL, van Vught LA et al. Likelihood of infection in patients with presumed sepsis at the time of intensive care unit admission: a cohort study. *Crit Care.* 2015 Sept 7;19(1):319.

7. Minderhoud TC, Spruyt C, Huisman S et al. Microbiological outcomes and antibiotic overuse in Emergency Department patients with suspected sepsis. *Neth J Med.* 2017 Jun;75(5):196-203.

8. Morley GL, Wacogne ID. UK recommendations for combating antimicrobial resistance: a review of 'antimicrobial stewardship: systems and processes for effective antimicrobial medicine use' (NICE guideline NG15, 2015) and related guidance. *Arch Dis Child Educ Pract Ed.* 2017.

9. Evans L, Rhodes A, Alhazzani W et al. Surviving Sepsis Campaign: International Guidelines for Management of Sepsis and Septic Shock 2021. *Critical that medicine.* 2021 ;49(11):e1063-e143.

10. Heffner AC, Horton JM, Marchick MR, Jones AE. Etiology of illness in patients with severe sepsis admitted to the hospital from the emergency department. *Clinical infectious diseases: an official publication of the Infectious Diseases Society of America.* 2010 Mar15;50(6):814-20.

11. Trzeciak S, Cymbal I, Phillip Dellinger R et al. Resuscitating the microcirculation in sepsis: the central role of nitric oxide, emerging concepts for novel therapies, and challenges for clinical trials. *Acad Emerg Med.* 2008 May;15(5):399-413.

12. Martin GS, Mannino DM, Eaton S, Moss M. The epidemiology of sepsis in the United States from 1979 through 2000. *The New England journal of medicine.* 2003 Apr 17;348(16):1546-54.

13. Dancour MA, Vaz JL, Bottino DA, Bouskela E. Nailfold videocapillaroscopy in patients with systemic lupus erythematosus. *Rheumatology international.* 2006 May;26(7):633-7.

14. Haak E, Haak T, Grozinger Y et al. The impact of contralateral cooling on skin capillary blood cell velocity in patients with diabetes mellitus. *J Vasc Res.* 1998 Jul-Aug;35(4):245-9.

15. Halfoun VL, Pires ML, Fernandes TJ et al. Videocapillaroscopy and Diabetes mellitus: area of transverse segment in nailfold capillar loops reflects vascular reactivity. *Diabetes research and clinical practice.* 2003 Sept;61(3):155-60.

16. Scardina GA, Carini F, Messina P. Oralo capillarscopy: a new diagnostic method. *Reumatismo.* 2005 Dec;57(4):295-304.

17. Scardina GA, Messina P. Microvascular characteristics of the human filiform papillae: a videocapillaroscopic study. *Ann Anat.* 2006 Mar;188(2):183-6.

18. Scardina GA, Ruggieri A, Messina P. Oral microcirculation observed in vivo by videocapillaroscopy: a review. *Journal of oral science.* 2009 Mar;51(1):1-10.

19. Tudor A, Musat A, Teleianu C et al. Aspects of nailfold capillaroscopy in children and young people with rheumatic disease. *Romanian journal of internal medicine = Revue roumaine of internal medecine.* 2004;42(1):211-5.

20. Bellavia F, Cacioppo A, Lupascu CA et al. A non-parametric segmentation methodology for oral videocapillaroscopic images. *Comput Methods Programs Biomed.* 2014 May;114(3):240-6.

21. Demirbas A, Elmas OF, Demirbas GU et al. Potential Utility of Oral Mucosal Capillaroscopy as an Indicator of Microvascular Damage in Behcet Disease: A Preliminary Study. *Dermatol Pract Concept.* 2021 Sep 1;11(4):e2021116.

22. Vermassen J, Decruyenaere J, De Bus L et al. Characteristics of Sepsis-2 septic shock patients failing to satisfy the Sepsis-3 septic shock definition: an analysis of real-time collected data. *Ann Intensive Care.* 2021 Oct 30;11(1):154.

23. Kalil AC. Sepsis and Septic Shock. *Fireplace Breathe Crit Care Med.* 2021;42(5):639-40.

24. Li X, Xu X, Xie F et al. A Time-Phased Machine Learning Model for Real-Time Prediction of Sepsis in Critical Care. *Critical that medicine.* 2020 Oct;48(10):e884-e8.

25. Hernandez G, Castro R, Bakker J. Capillary refill time: the missing link between macrocirculation and microcirculation in septic shock? *J Thorac Dis.* 2020 Mar;12(3):1127-9.

26. Scardina GA, Cacioppo A, Pisano T, Messina P. In vivo evaluation of labial microcirculation in diabetics: a comparison with healthy subjects. *Panminerva Med.* 2011 Jun;53(2):81-5.

27. Scardina GA, Fuca G, Messina P. Microvascular characteristics of the human interdental papilla. *Anat Histol Embryol.* 2007 Aug;36(4):266-8.

28. Scardina GA, Messina P. Study of the microcirculation of oral mucosa in healthy subjects. *Ital J Anat Embryol.* 2003 Jan-Mar;108(1):39-48.