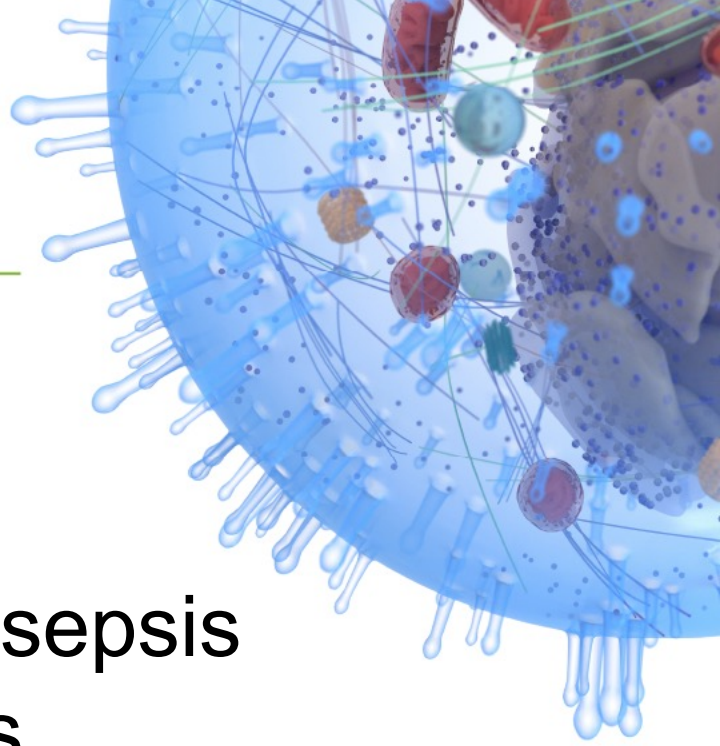
ICoMI VIRTUAL
2nd International Congress of
Micro-immunotherapy
June 2-4, 2022



Expression patterns of monocyte subsets in sepsis patients with and without metabolic disorders

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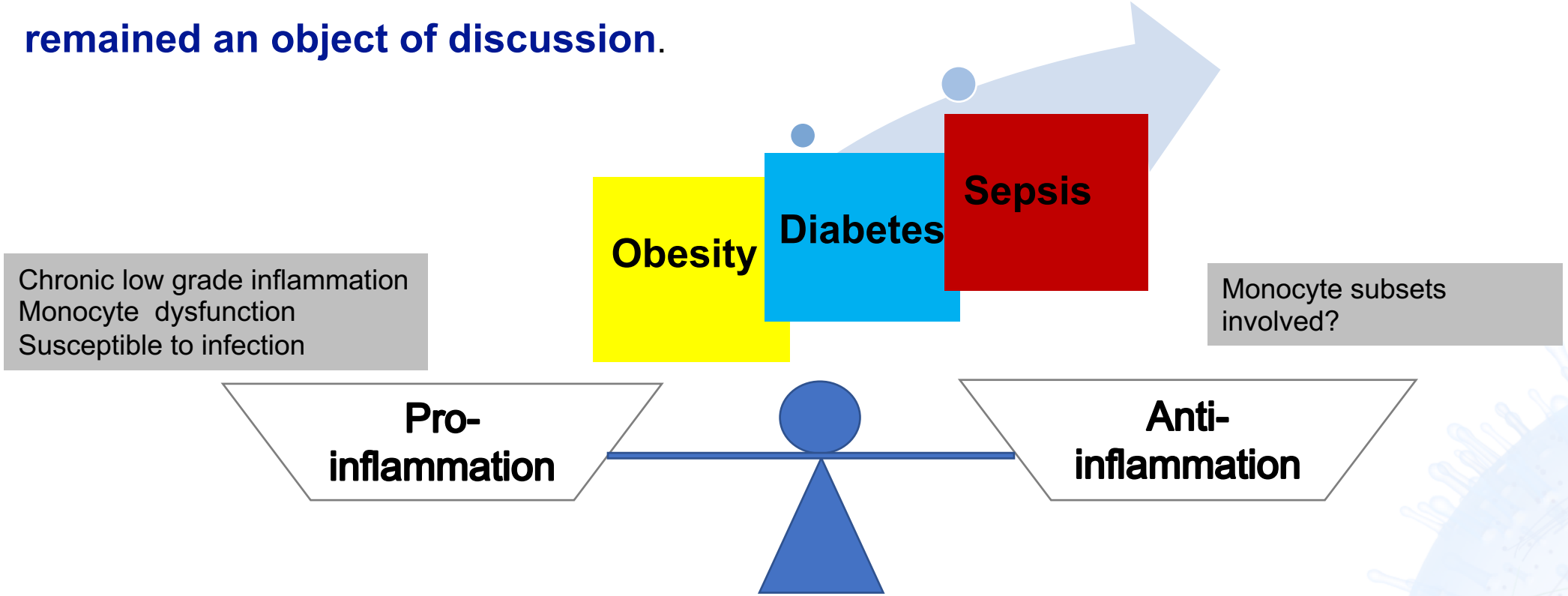
- **Obesity, diabetes mellitus (DM), sepsis** are major public health problems worldwide, which
  - linked to monocyte/macrophage dysfunction.

(Fingerle G etal., 1993; Ziegler-Heitbrock L, 2007; Singer et al., 2016; Grün JL et al., 2018)

- However, phenotypic modulation of monocyte subsets in sepsis with obesity and diabetes **remained an object of discussion**.

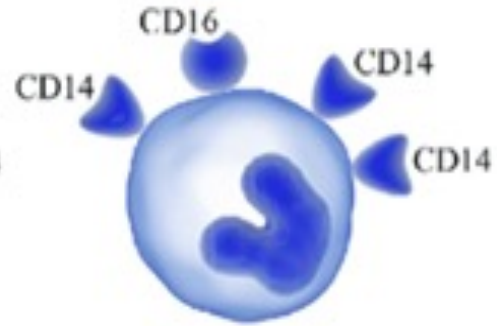


Human

Classical (CL) Monocyte

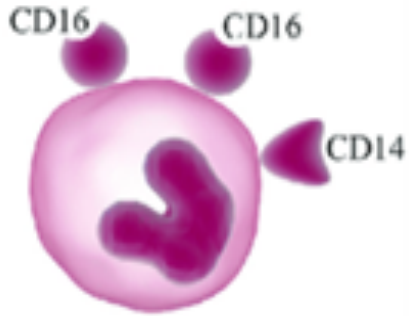
CD14++ CD16-

Intermediate (INT) Monocyte



CD14++ CD16+

Non-classical (NCL) Monocyte



CD14+ CD16++

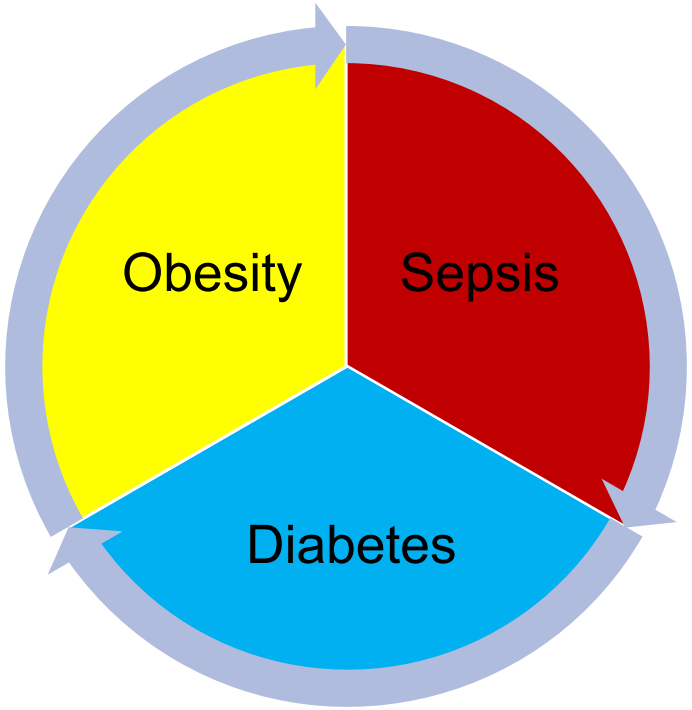
Markers	CD33++ CD163++ HLA-DR +	CD33++ CD163++ HLA-DR ++	CD33+ - HLA-DR ++
Cytokines	IL-10	TNF, IL-1β, IL-12, ROS,NO	TNF, IL-1β, ROS,NO
Function	Phagocytosis and regulation of inflammation	Pro-inflammation	Patroling to sense tissue injury, repair damage and removed dead cells, aging, viral infection

https://www.researchgate.net/figure. Ziegler-Heitbrock, 2010; Passlick et al., 1989; Fingerle et al.,1993; René etal.,2018; Swiew-Min et al.,2019.

Overview of the current work

Purpose:

- to characterize the phenotypes of monocyte subsets in septic patients with obesity and diabetes mellitus.



Monocytes

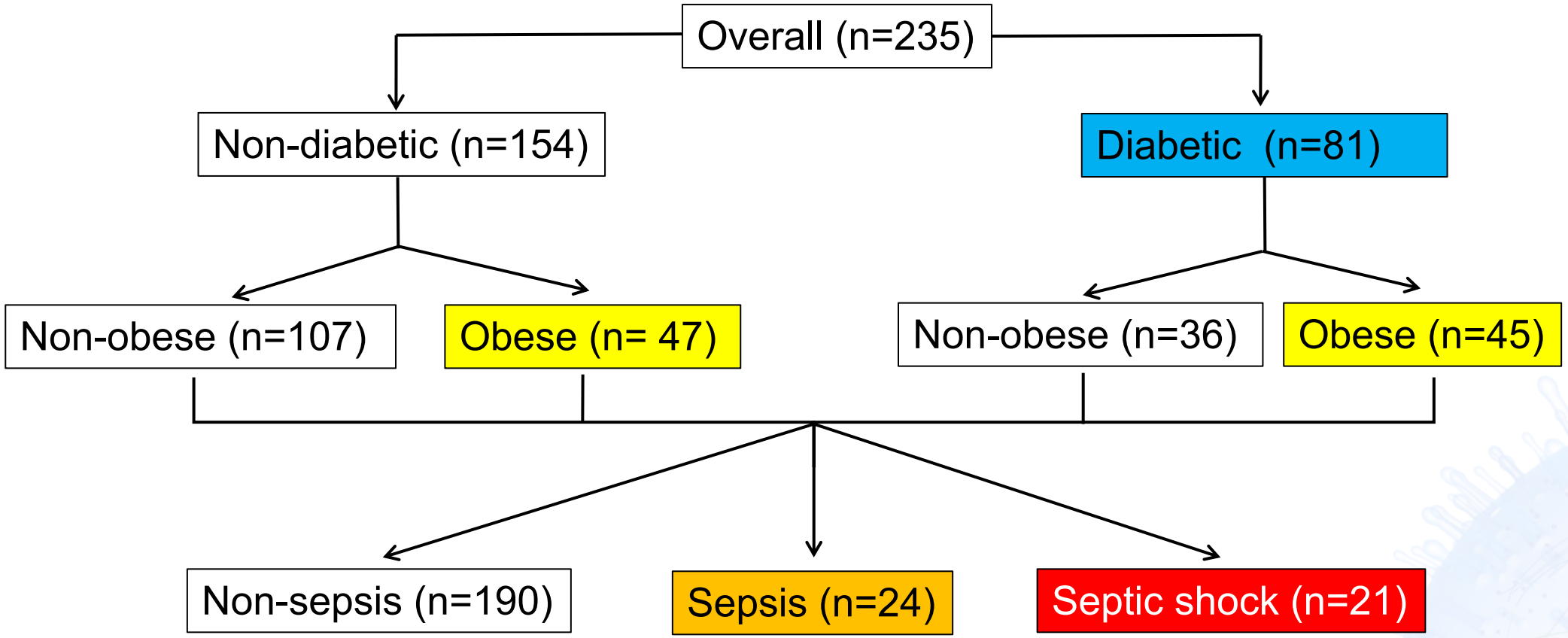
Questions aimed to answer:

- How do monocyte subsets differ between non-sepsis, sepsis, and septic shock in critically ill (ICU) patients?
- Do monocyte subsets differ between non-sepsis, sepsis, and septic shock in ICU patients with and without diabetes?
- What is the effect of obesity on monocyte subsets between non-sepsis, sepsis, and septic shock in ICU patients with and without diabetes?

Patients

- 235 critically ill patients from Intensive Care Unit of Ulm University Hospital.

(This study were approved by the local ethics committee of Ulm University.)

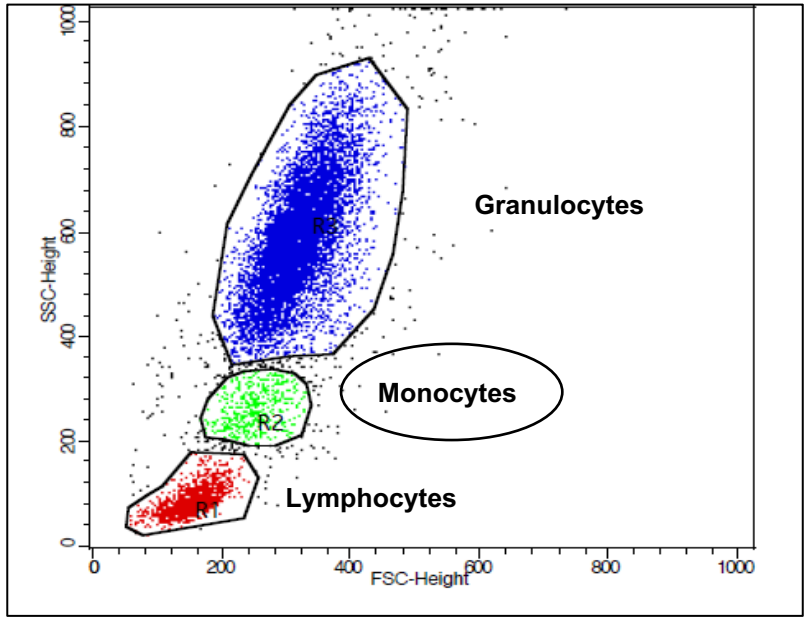


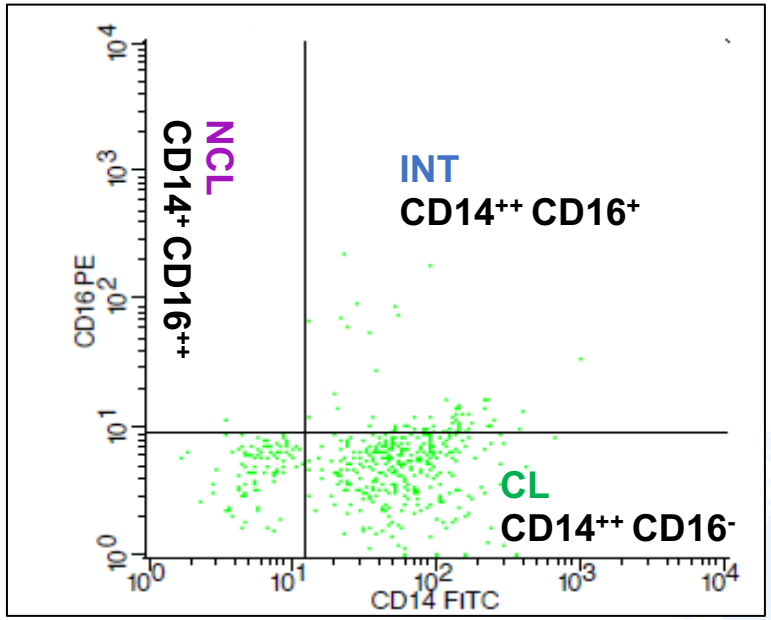
Methods

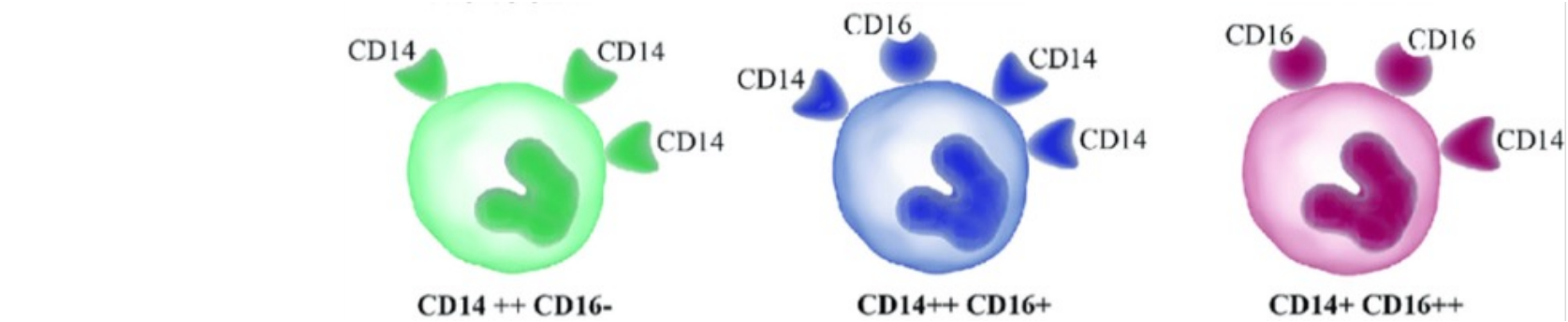
➢ Flow cytometry

- Well mixed blood sample was labeled with the antibodies (CD14, CD16, CD33, CD163, HLA-DR) and 1µg/ml for 30 minutes at 4 C°in dark; wash 2 times with 1x PBS; measured with BD flow cytometer
- Gating stategy for monocyte subsets (**CD14-CD16 classification**)

➢ Statistical analysis: *Mann Whitney test or Kruskal Wallis test, Spearman rank correlation analysis.*



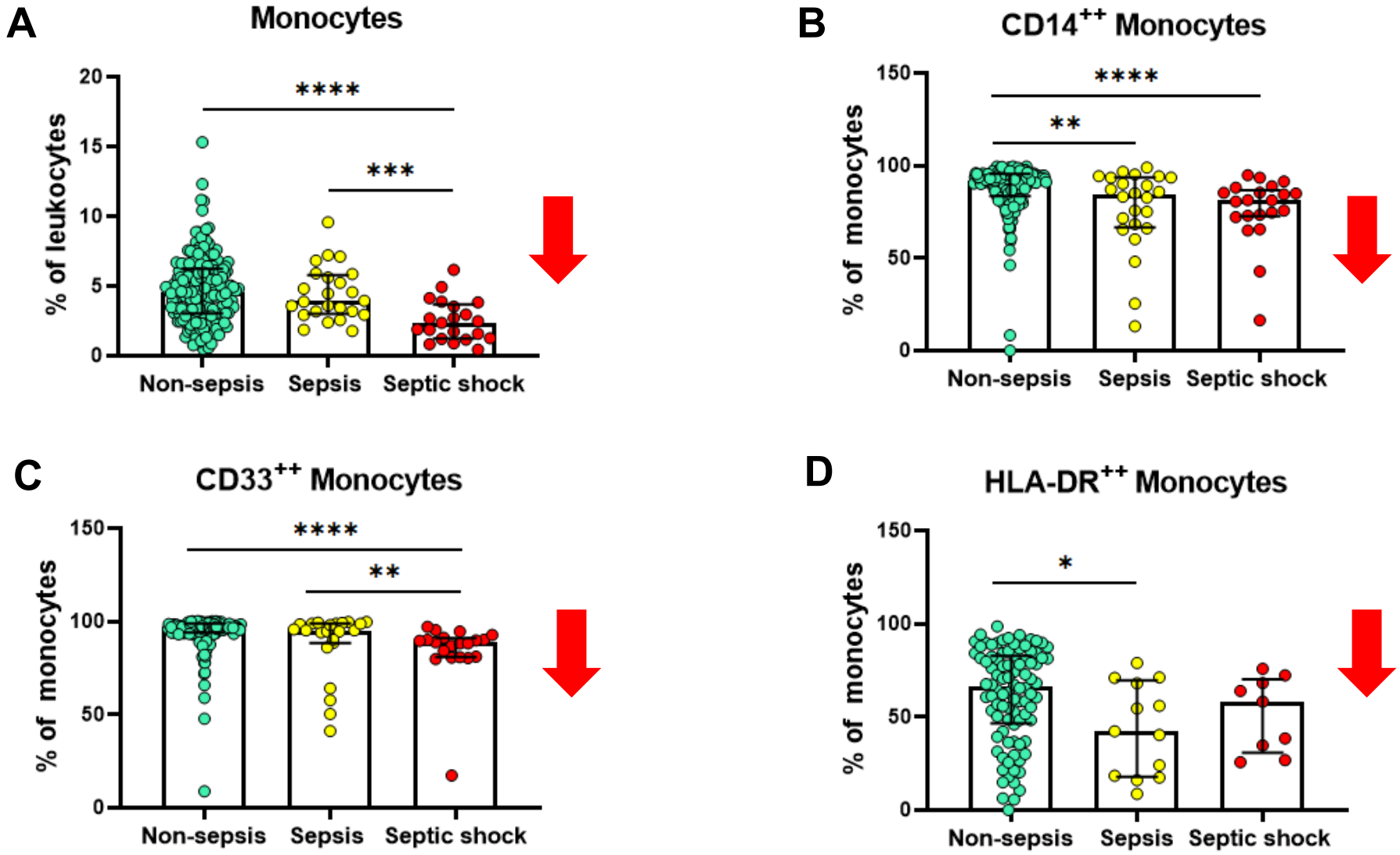




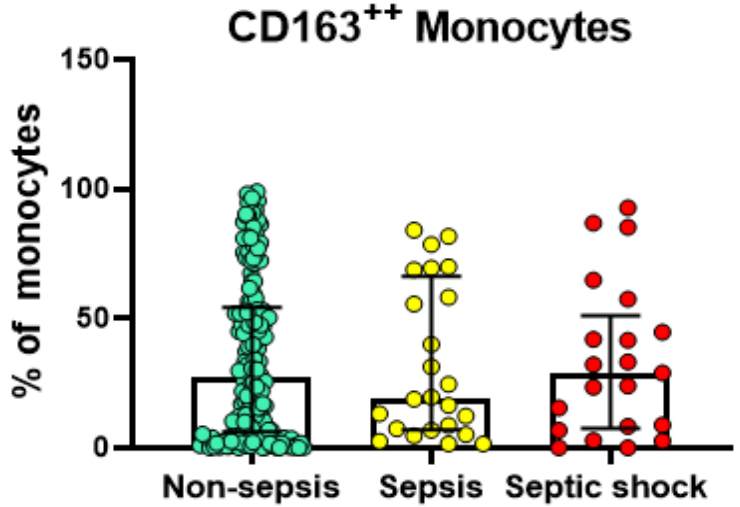
Results 1

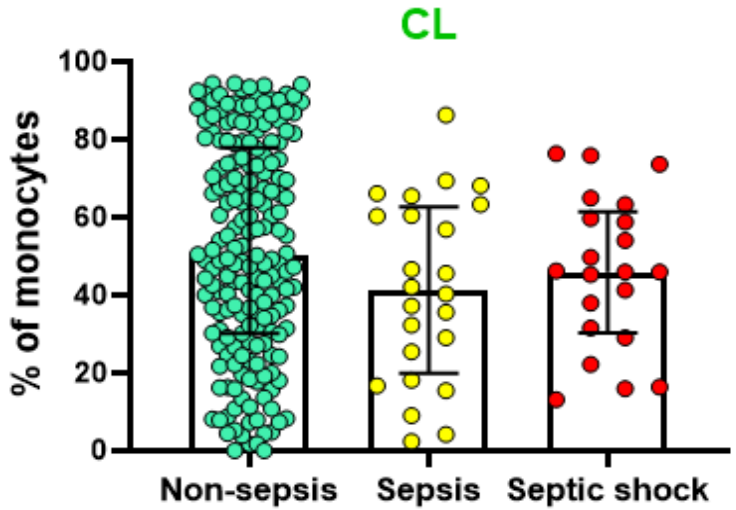
Differences of monocyte subsets in all ICU patients between

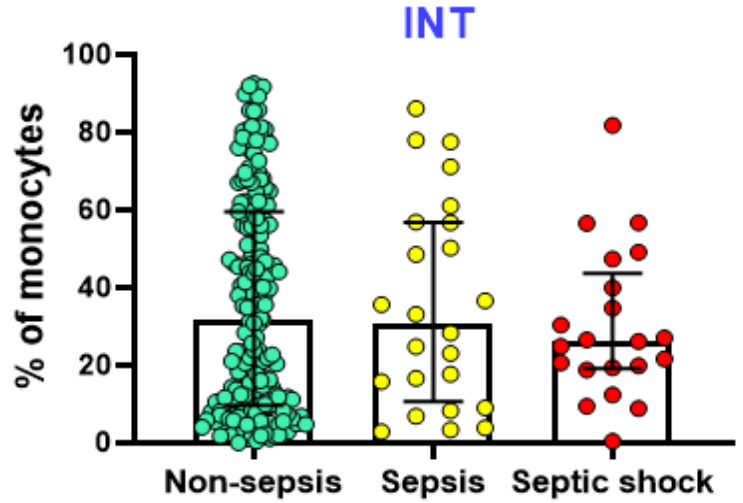
- Non-sepsis
- Sepsis
- Septic shock

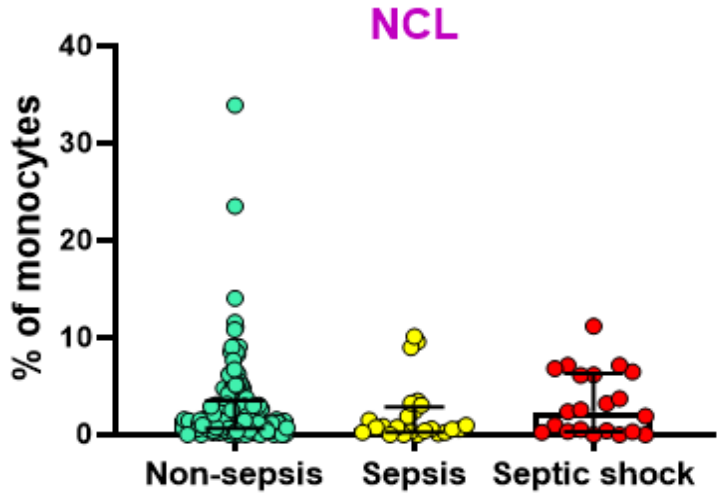


- Septic shock patients **decreased** monocytes%, CD14^{++} % and CD33^{++} % (CL and INT).
- Sepsis patients have **lower** HLA-DR $^{++}$ % (INT and NCL).

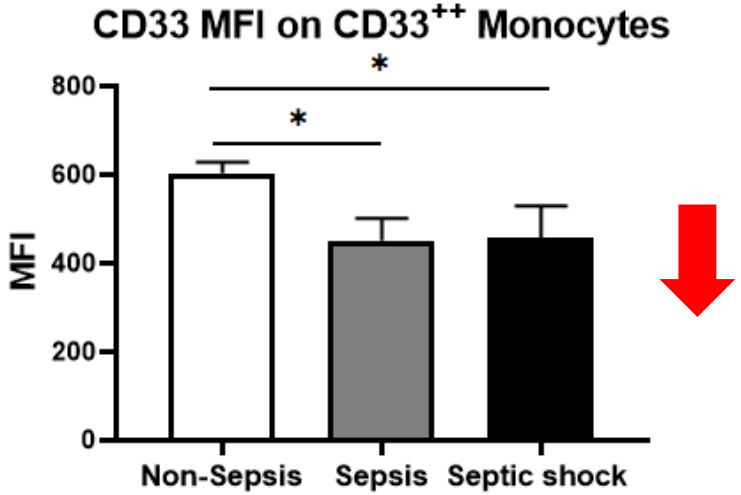


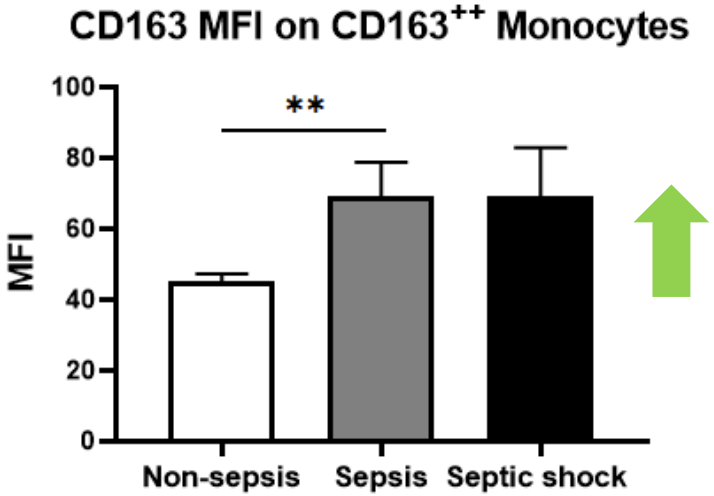


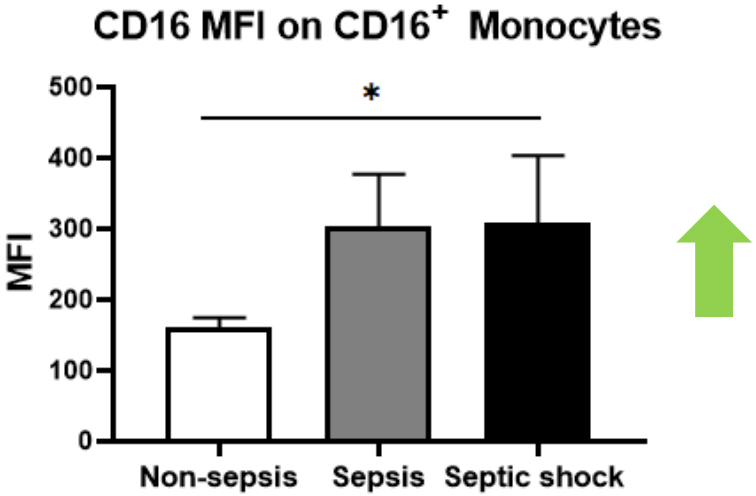




- **No significant** differences of three subsets and CD163^{++} subsets (CL + INT) were found between non-sepsis, sepsis and septic shock in all ICU patients.

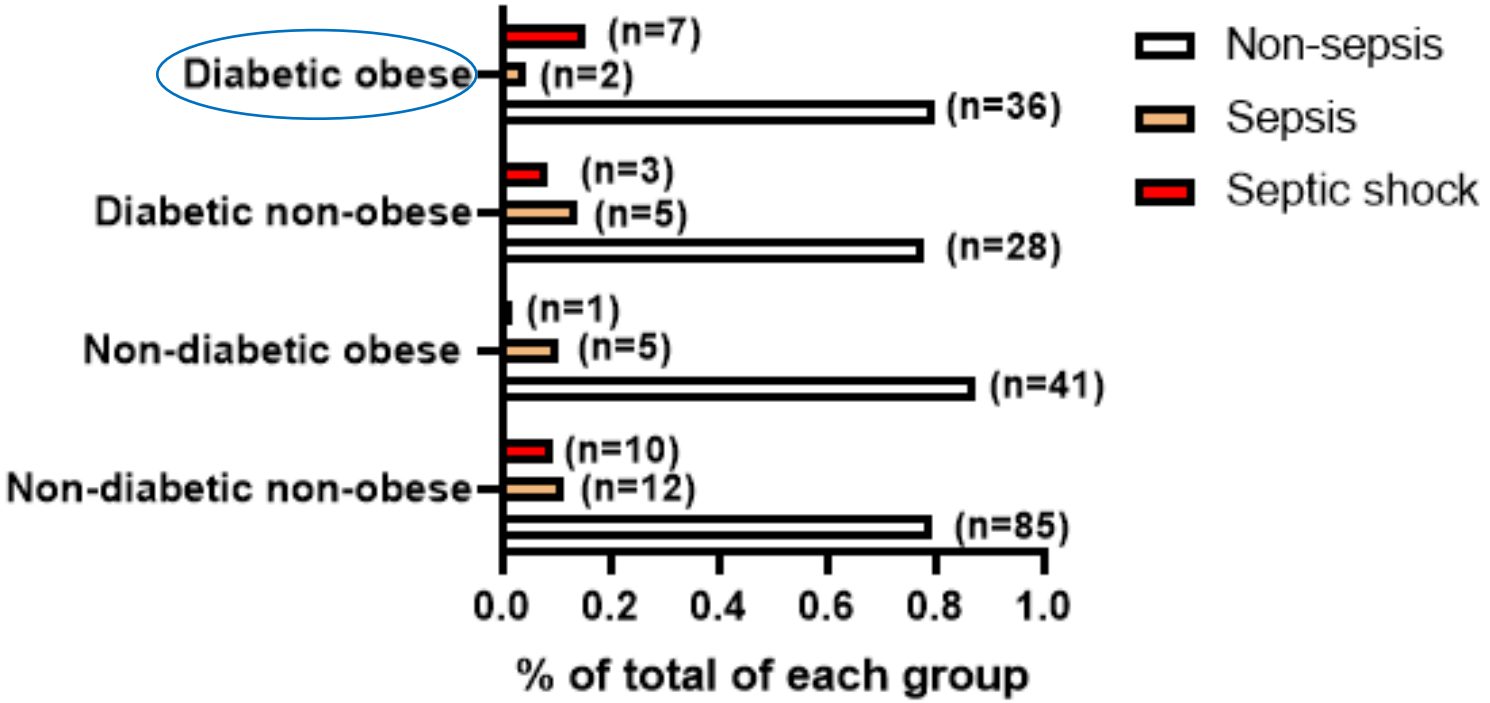






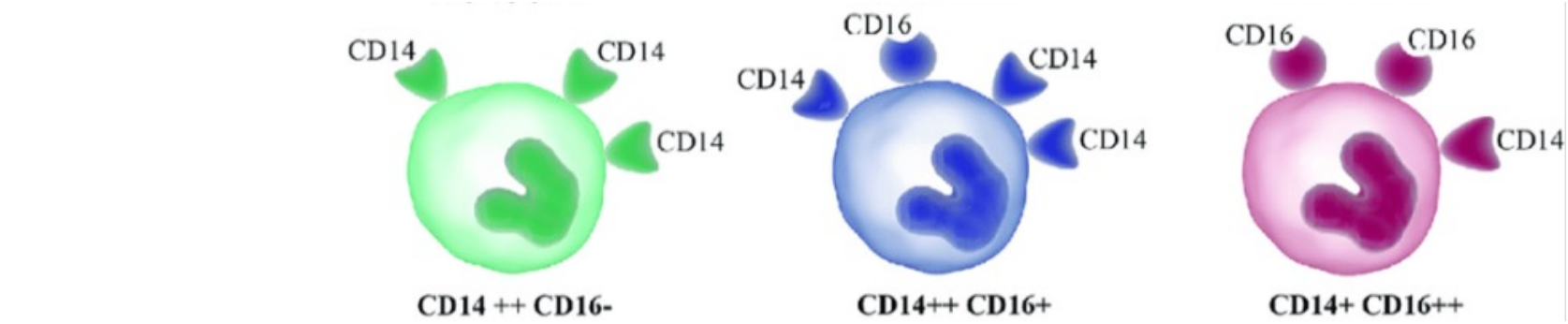
- Septic patients display **higher** expression of CD16, CD163, but **lower** CD33 expression than non-sepsis patients.

Relative amounts of septic patients



- The frequency of **septic shock** is significantly higher in **obese and diabetic** patients than that in obese and non-diabetic patients (p <0.05).

Are there different effect of obesity and diabetes on monocyte subsets in ICU sepsis patients versus non-sepsis patients?



CL INT NCL

Results 2

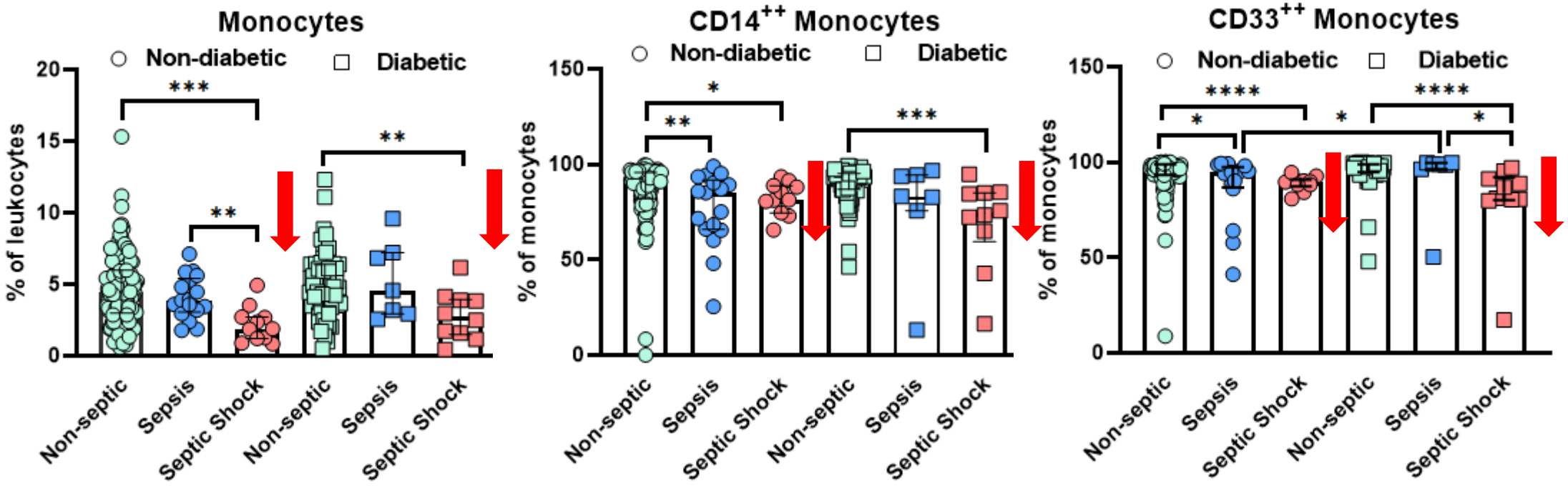
Differences of monocyte subsets in ICU patients with and without diabetes

Non-sepsis

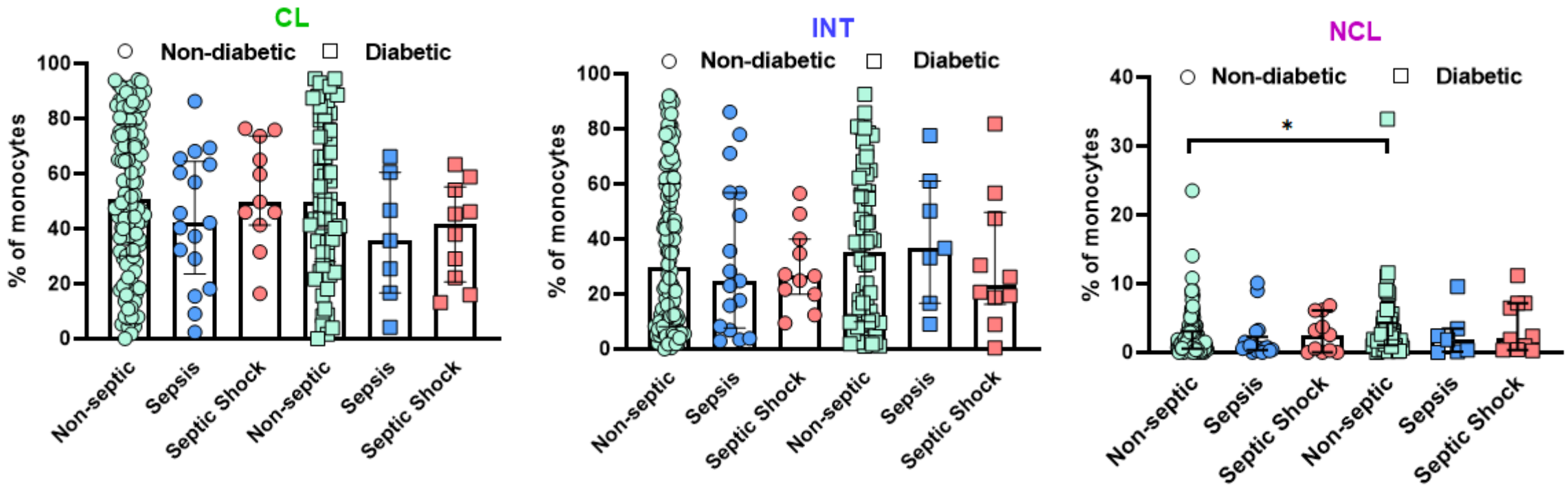
Sepsis

Septic shock

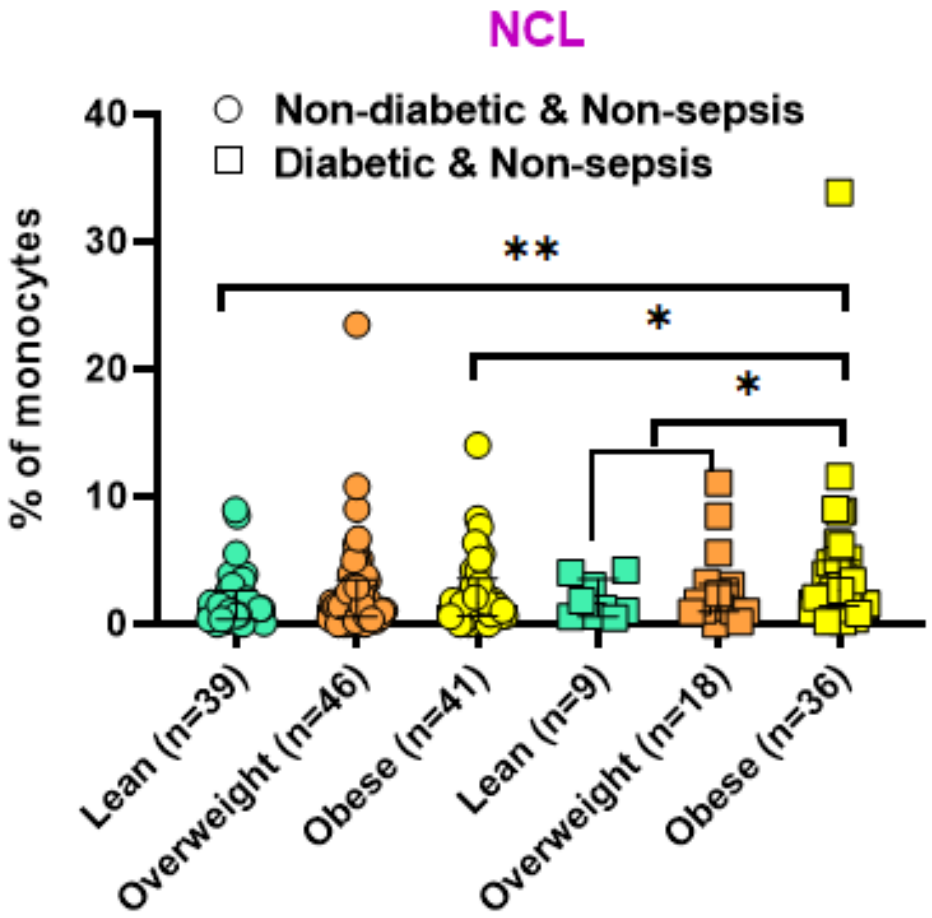
Alterations of monocyte subsets in ICU patients **with and without diabetes**



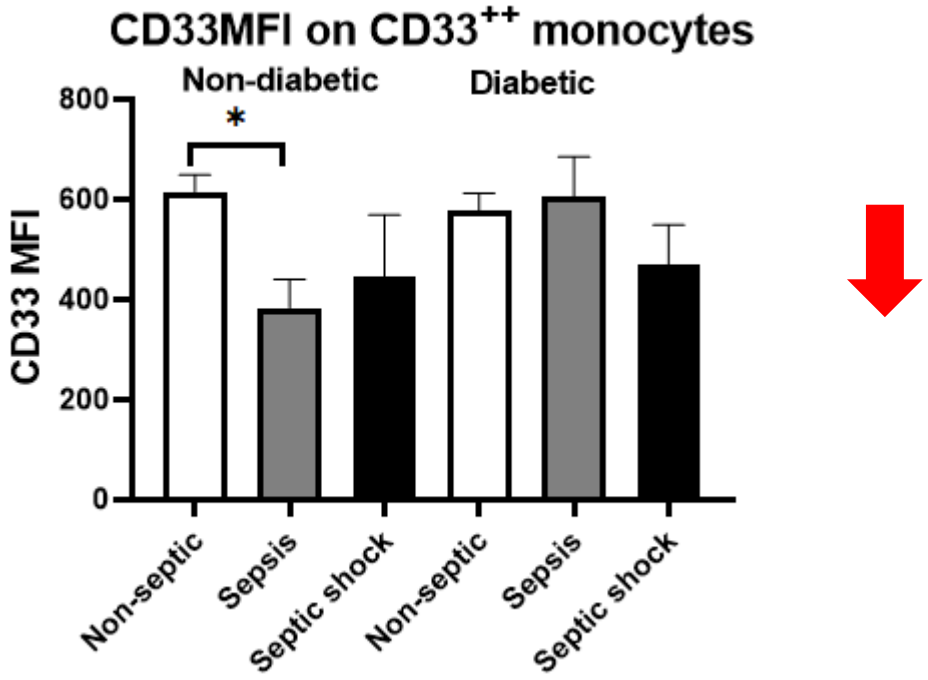
- In both non-diabetic and diabetic patients, **septic shock** patients present with **lower** monocytes, CD14^{++} and CD33^{++} monocytes (**CL** and **INT**).

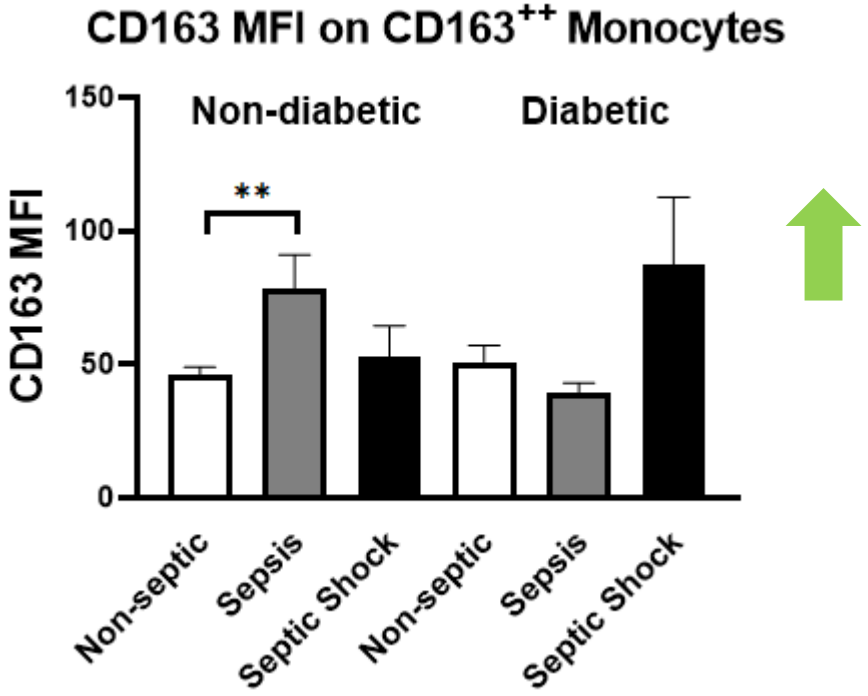


- **Diabetic** sepsis patients have **lower** **CL**
- **Diabetic** non-septic and septic shock patients present with **higher** **NCL** relative to non-diabetic patients.



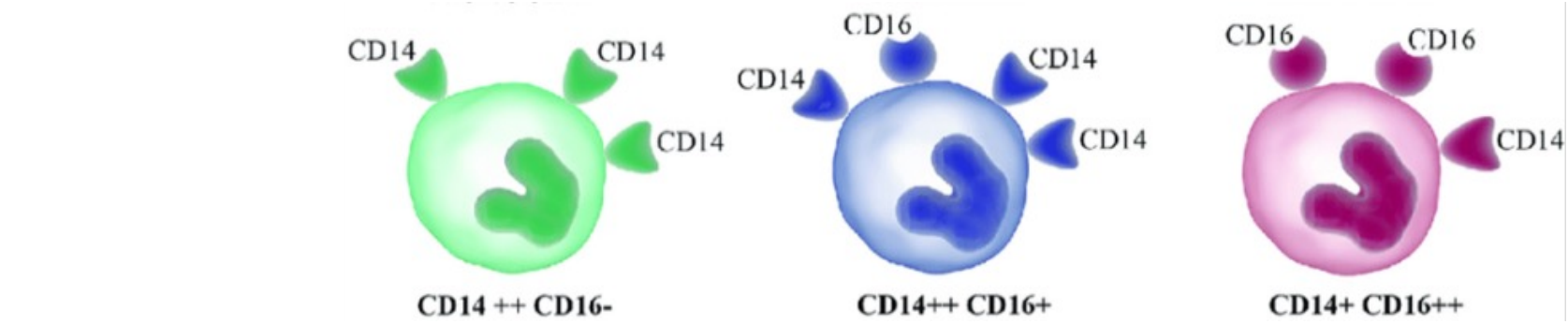
- **Diabetic** obese non-septic patients present with prominently **higher** **NCL** monocytes.





- **Diabetic** septic shock patients display lower CD33 expression

- **Diabetic** septic shock patients display higher CD163 expression



Results 3

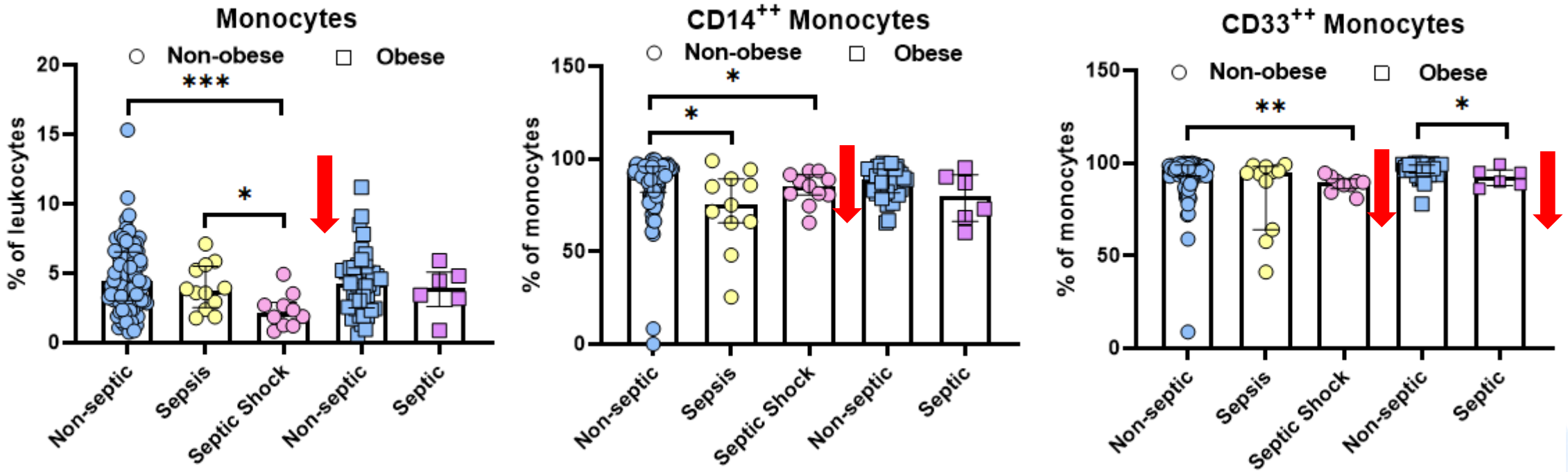
Effect of obesity on monocyte subsets in ICU patients with and without diabetes

Non-sepsis

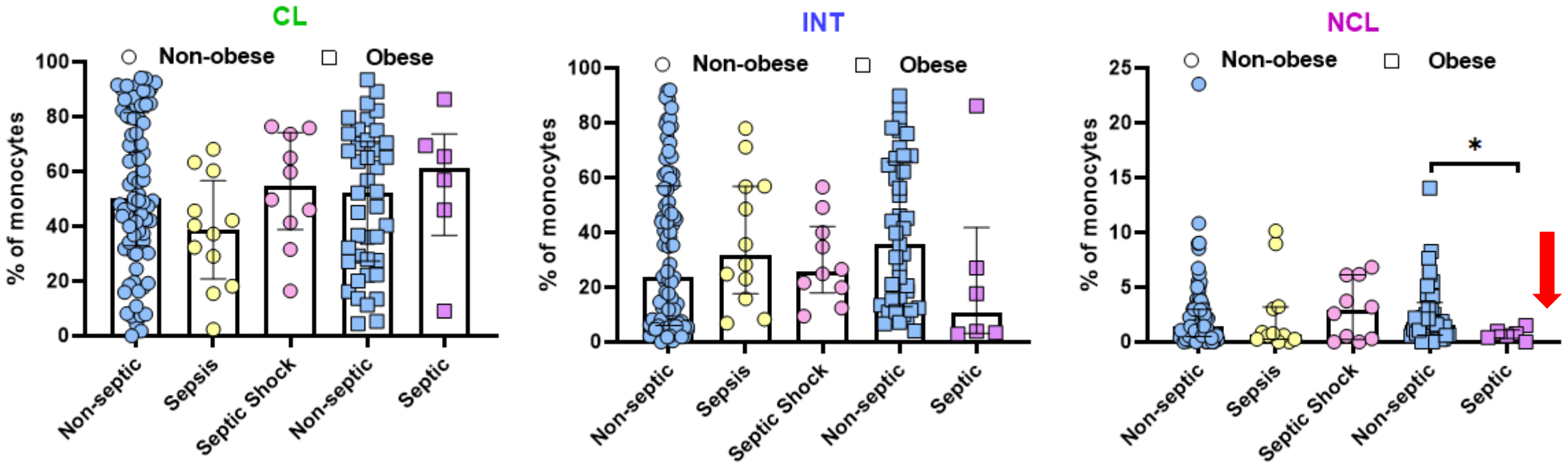
Sepsis

Septic shock

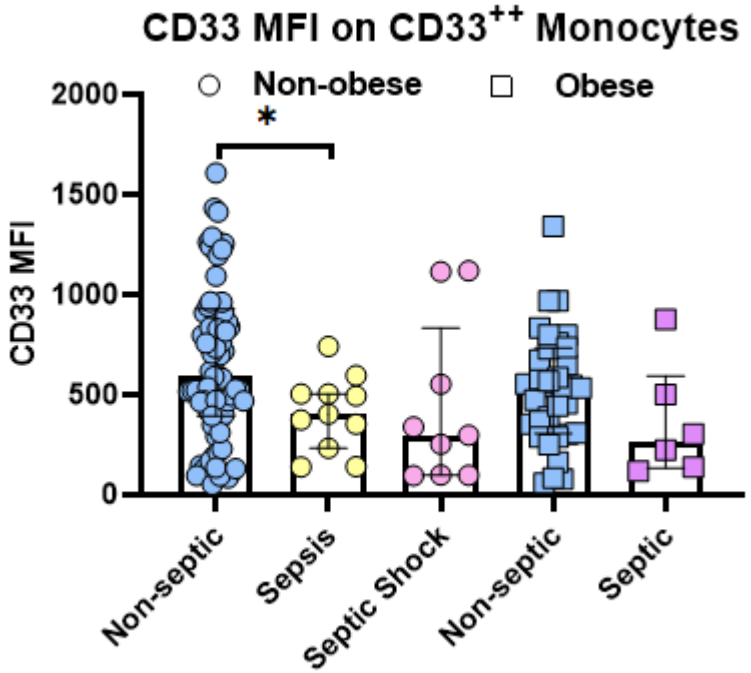
Effect of obesity on monocyte subsets in ICU septic patients without diabetes

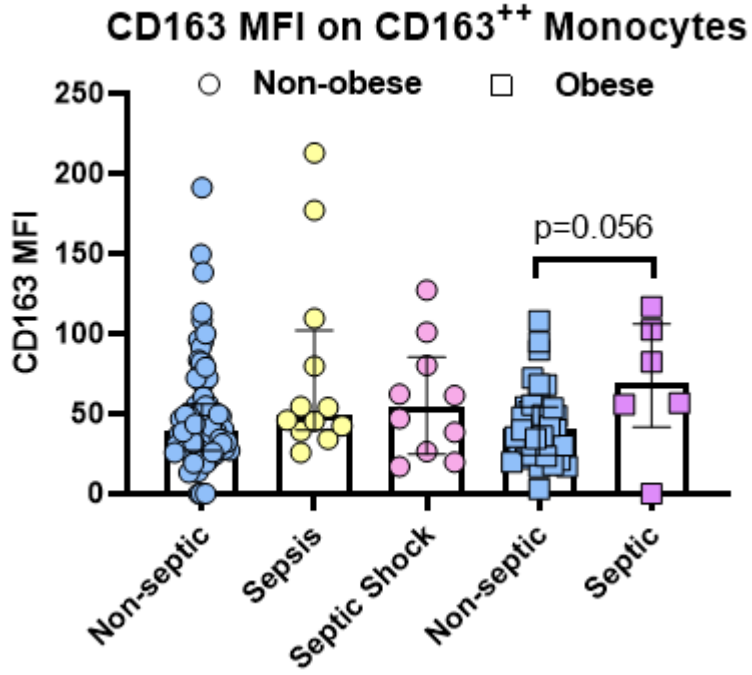


- **Obese** septic patients **did not** show a decrease of CD14^{++} monocytes.
- Both **obese** and **non-obese** septic patients have **decreased** CD33^{++} monocytes.

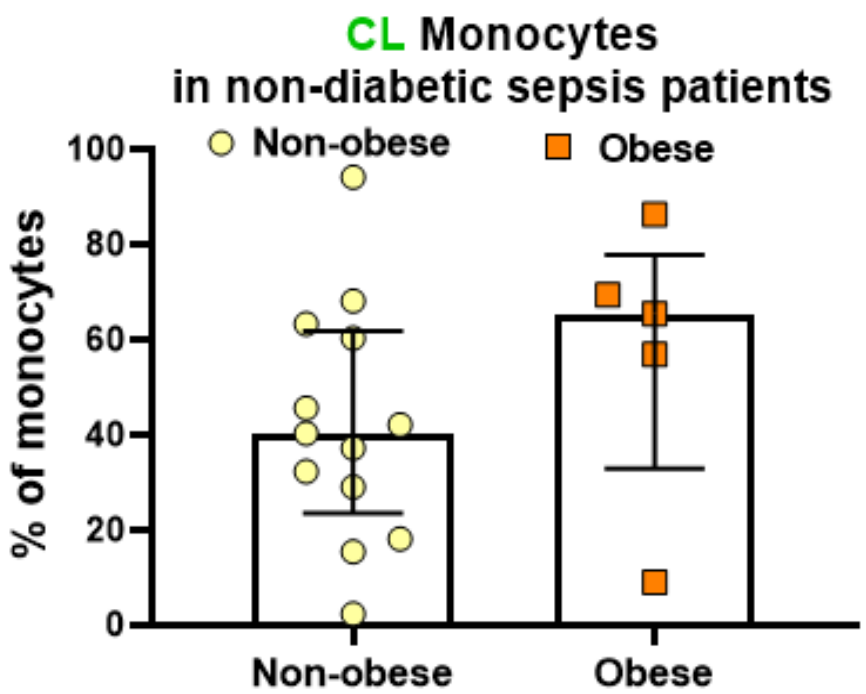


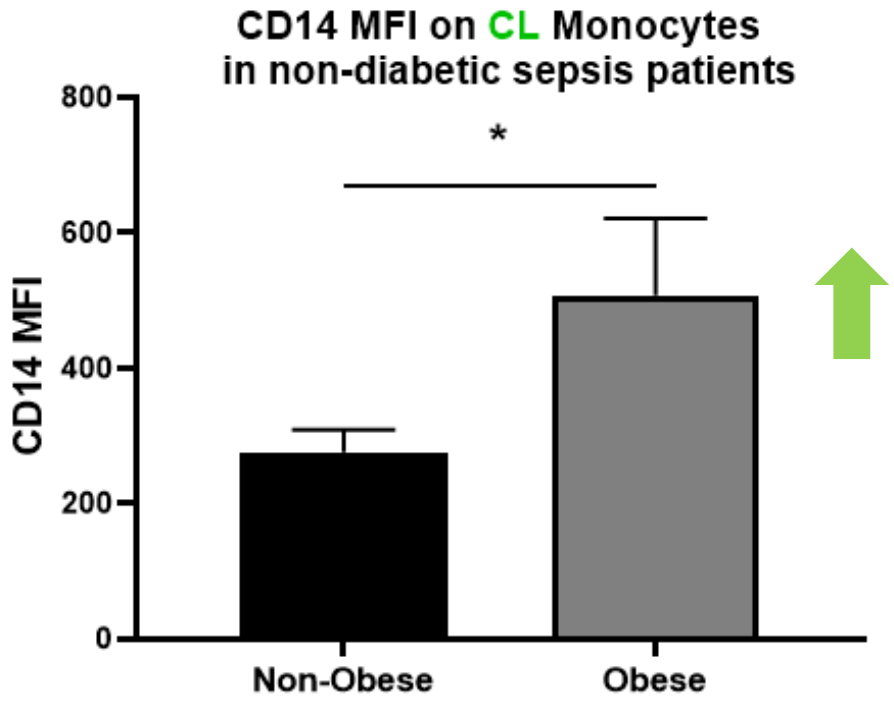
- **Obese** non-diabetic septic patients have significantly **lower NCL** monocytes.





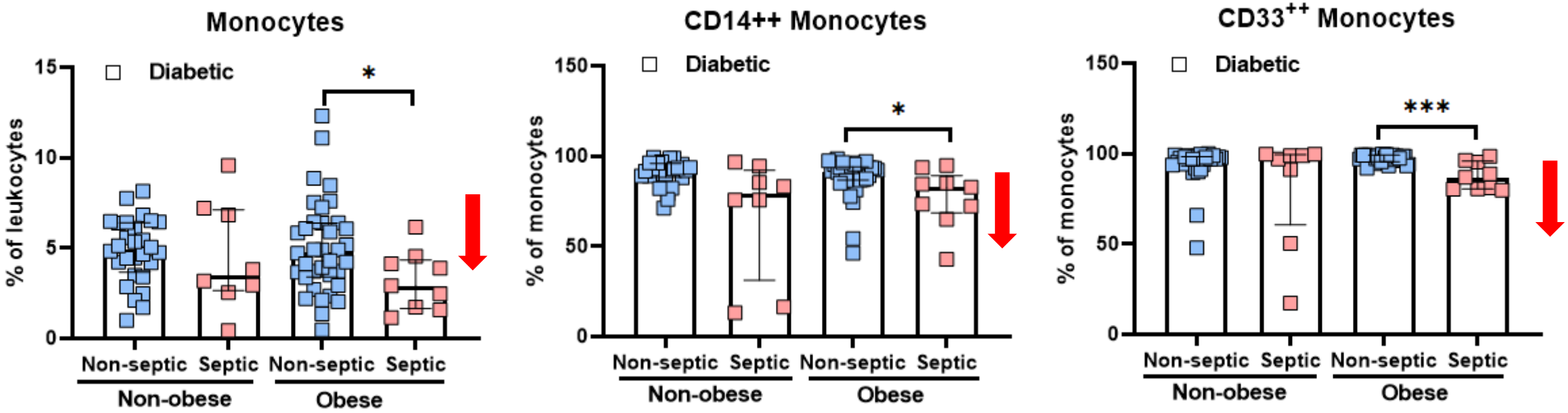
- **Obese** septic patients without diabetes **do not** differ in their expression of CD33 and CD163.



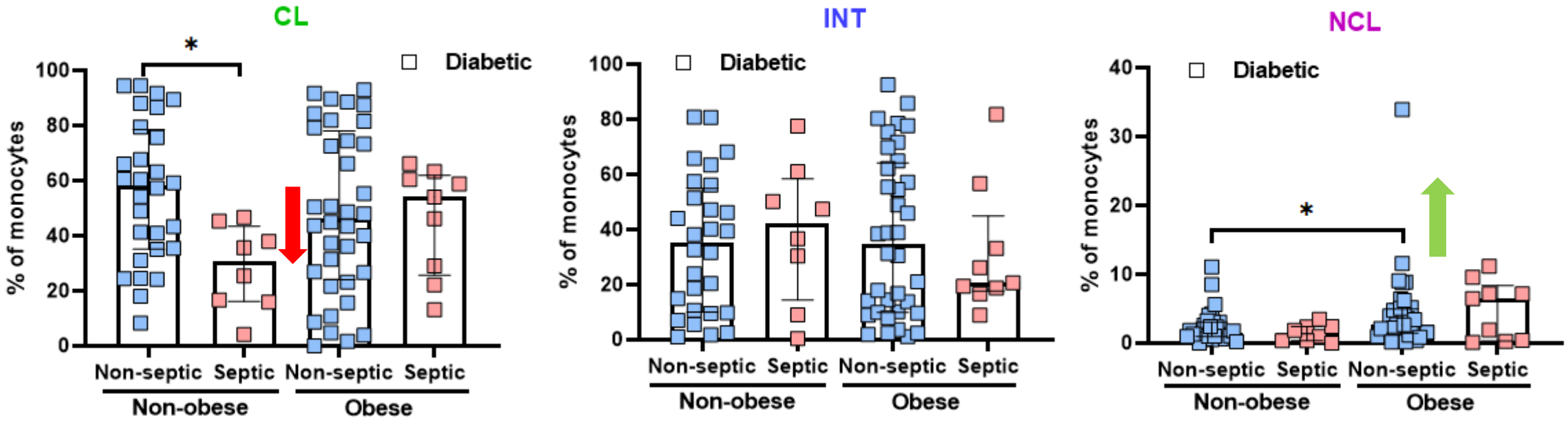


- Non-diabetic sepsis patients with **obesity** show relatively **higher** **CL** monocytes and significantly **increased** CD14 expression on **CL**.

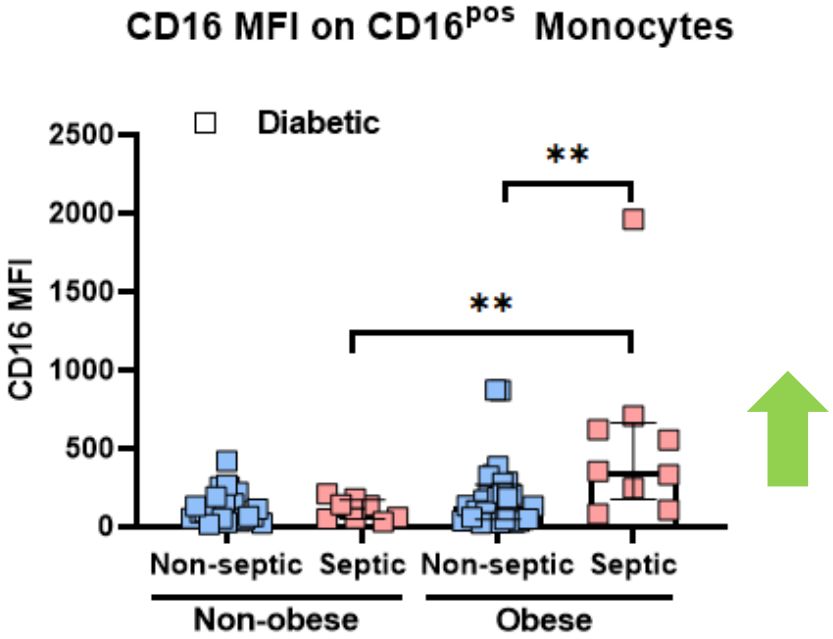
Effect of obesity on monocyte subsets in ICU septic patients with diabetes

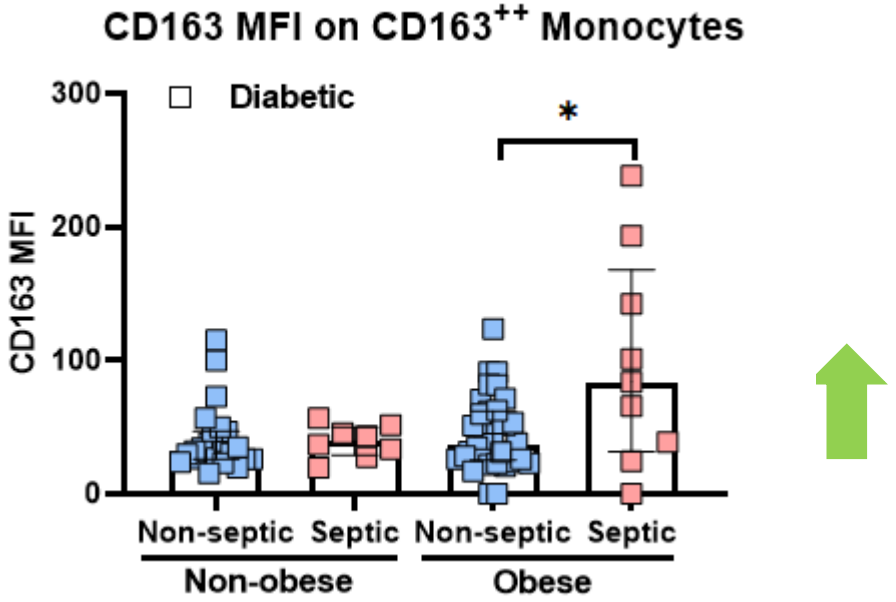


- **Diabetic obese septic** patients have significantly **lower** total monocytes, and $CD14^{++}$, $CD33^{++}$ monocytes.

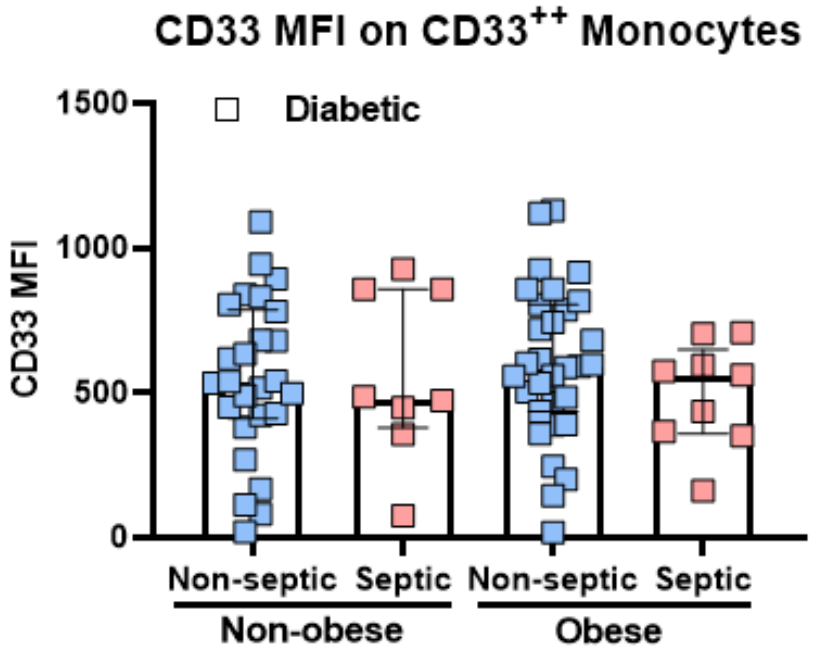


- **Diabetic obese septic** patients display **higher CL** and **NCL**, but **lower INT** monocyte counts.





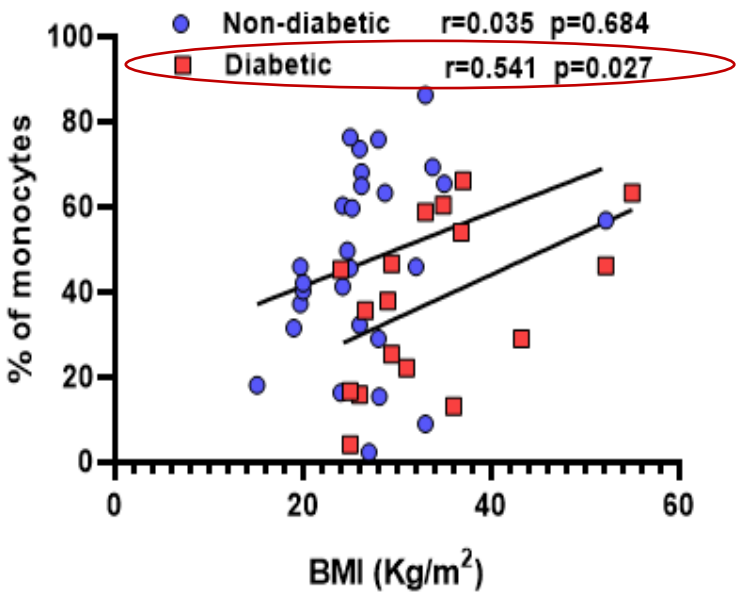
- **CD16** is highly **increased** in sepsis patients with obesity and diabetes
- **CD163** is more **increased** in sepsis patients with obesity and diabetes



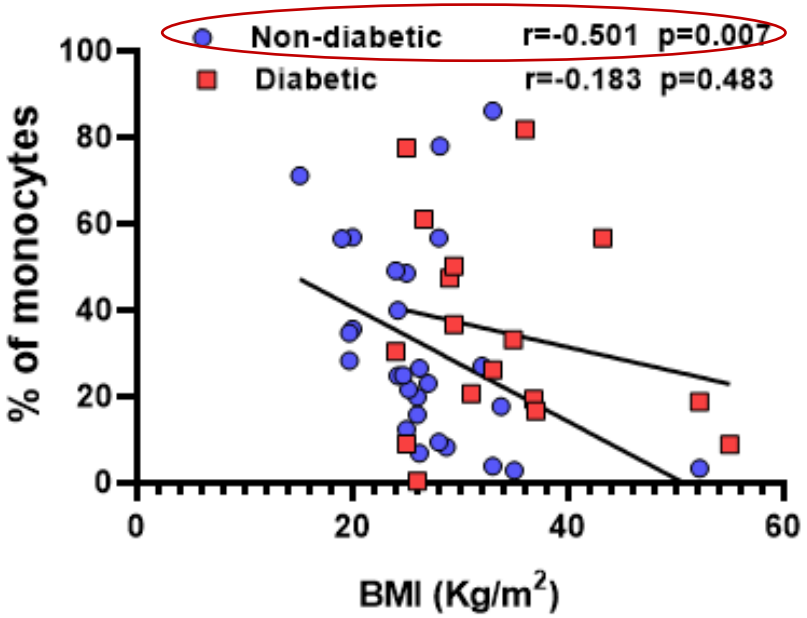
- **No differences** of CD33 expression were found between non-obese and obese patients.

Correlation analysis of monocytes with **BMI** in sepsis patients with diabetes

CL

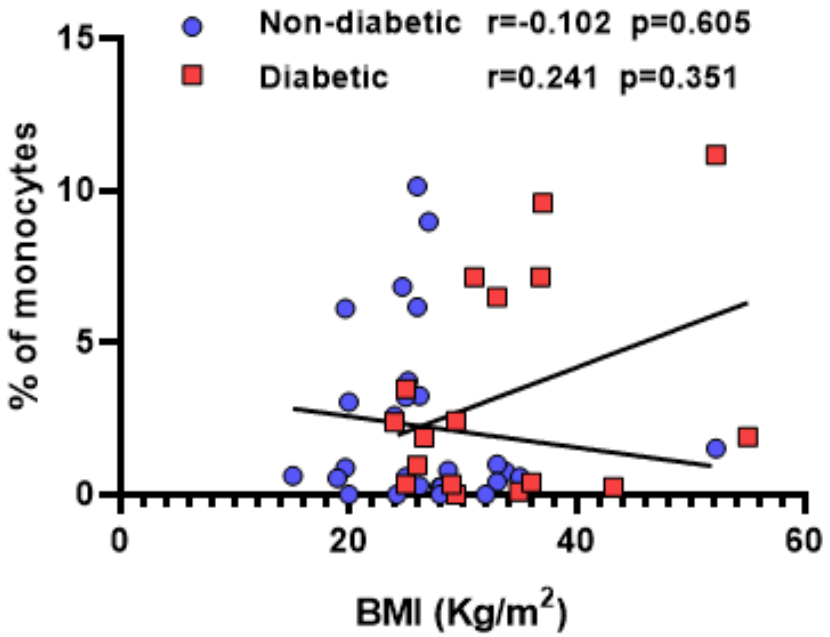


INT

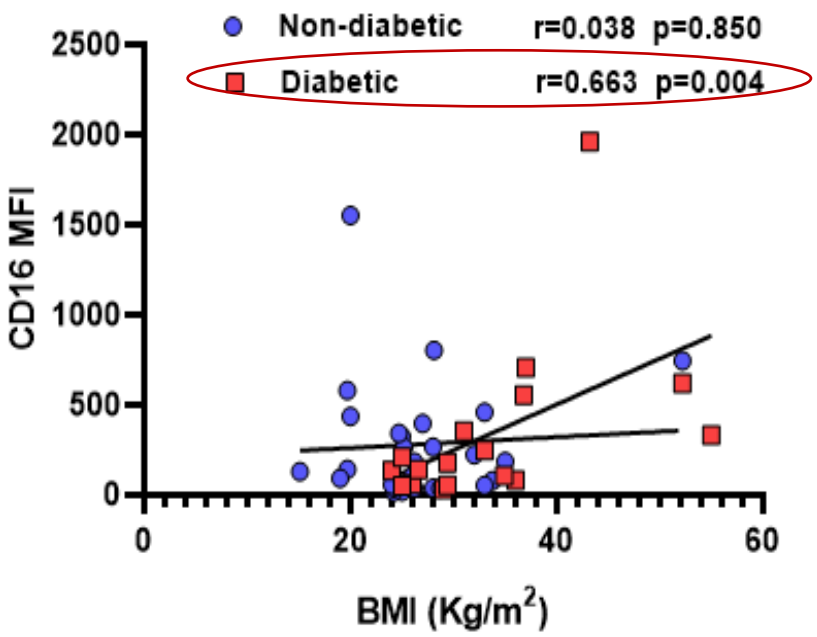


- **Increased CL** monocytes correlate with **BMI** in **diabetic** sepsis but not in non-diabetic patients
- **Lower INT** monocytes inversely correlate with **BMI** in **non-diabetic** sepsis but not in diabetic patiens

NCL

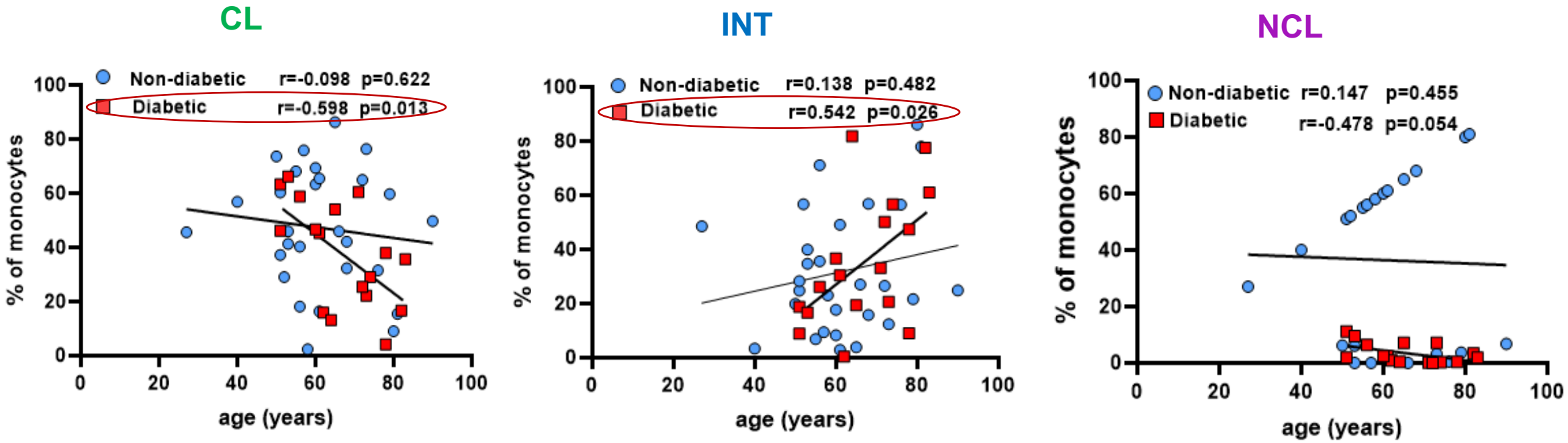


CD16^{pos} Monocytes



- **NCL no significant** correlation with BMI in diabetic and non-diabetic sepsis patients
- Increased **CD16 expression** on CD16^{pos} monocytes **positively** correlates with **BMI** in **diabetic** sepsis but not in non-diabetic patients

Correlation analysis of monocytes with age in sepsis patients with diabetes



- **CL inversely** correlate and **INT positively** correlate with **age** in **diabetic** sepsis patients but not in non-diabetic patients
- **NCL no significant** correlation with age in sepsis patients with and without diabetes

General discussion

- Depletion of **CL** implies impaired phagocytosis in critically ill patients with sepsis. (Serbina et al., 2008)
- **NCL** may play a role in obesity related inflammation in diabetic patients. (Poitou et al., 2011; Rogacev et al., 2010)
- Down-regulation of CD33 expression and $CD33^{++}$ subsets implies prolonged pro-inflammatory immunity during sepsis. (Orr et al., 2007; Paul et al., 2000)
- Upregulated expression of CD163 and CD16, and $CD16^{pos}$ subsets may reflect endotoxin tolerance in septic patients with diabetes and obesity. (Röszer, 2015; Zhang et al., 2014; Shalova et al., 2012)
- Limitations: Sample sizes in subgroups were quite small, but the results helped to design larger studies to understand immune dysregulation in patients with diabetes and obesity with sepsis.

Summary

- As a novel finding, we identified the selective diminution of $CD14^{++}$ (**CL**) and CD33 expression in monocytes of sepsis patients. This effect is more pronounced in diabetic patients progressing from sepsis to septic shock.
- Obese non-septic patients with diabetes have higher **NCL** monocytes.
- Obese septic patients with diabetes showed increased CD16 expression, as well as CD163.

Acknowledgements

Prof. Dr. Marion Schneider

Prof. Dr. Eberhard Barth

Prof. Dr. Benjamin Mayer

Prof. Dr. Tamas Röszer

Dr. Benedikt Zulajovic

Ronald Stitz (Medical student)

Pauline Stitz (Medical student)

Christian Scheiber (PhD student)

Chen Li (PhD student)

our Research Team…