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INTRODUCTION

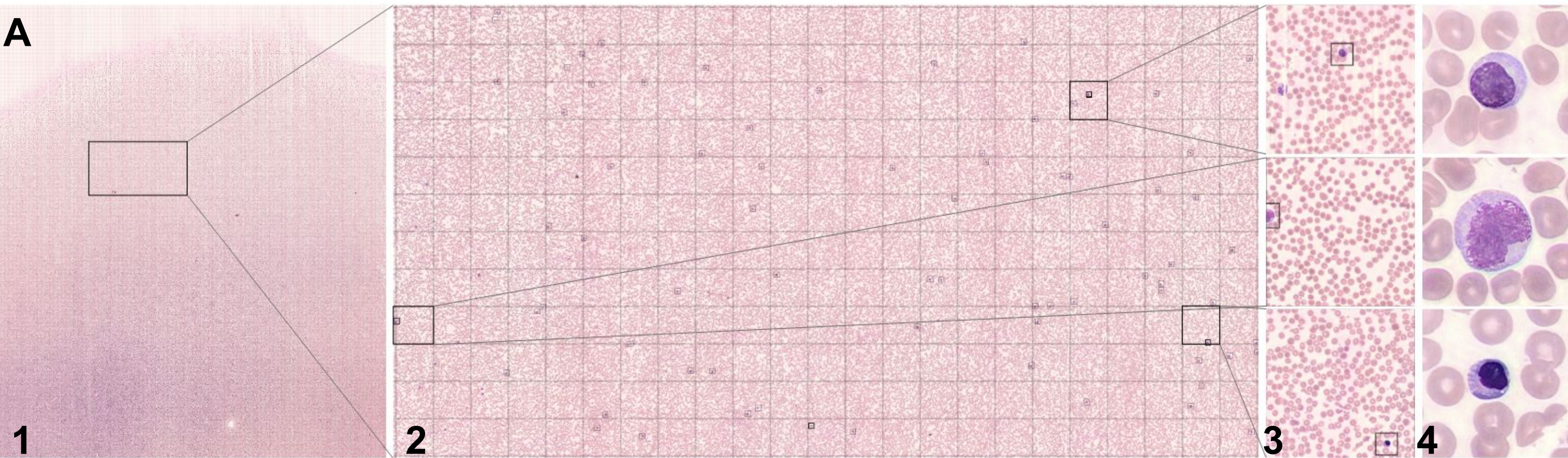
Chimeric Antigen Receptor T-cells (CAR-T) therapy becomes a preferable therapeutic approach in patients with relapsed and refractory (R/R) non-Hodgkin's large B cell lymphomas (LBCL) who failed ≥ 2 therapeutic regimens. However, there are no commercial assays for routine CAR-T measurements and clinical studies often employ PCR or FACS, both expensive and relatively complicated. Detection of CAR-T in peripheral blood smear (PBS) is challenging, mostly due to the lack of data regarding their morphology prior to transfusion and the low sensitivity of currently available technologies. As oppose to the currently available digital morphology, Scopio Labs Full Field Morphology (FFM) is a novel digital microscopy platform that provides high-resolution images combined with a full field analysis, incorporating artificial intelligence capabilities, thereby enabling the detection and classification of rare cells in PBS, including CAR-T cells.

OBJECTIVES

The dynamics of CAR-T quantity and subtypes in PBS were evaluated using the FFM platform and correlated with responsiveness to treatment and treatment-related toxicities.

METHODS

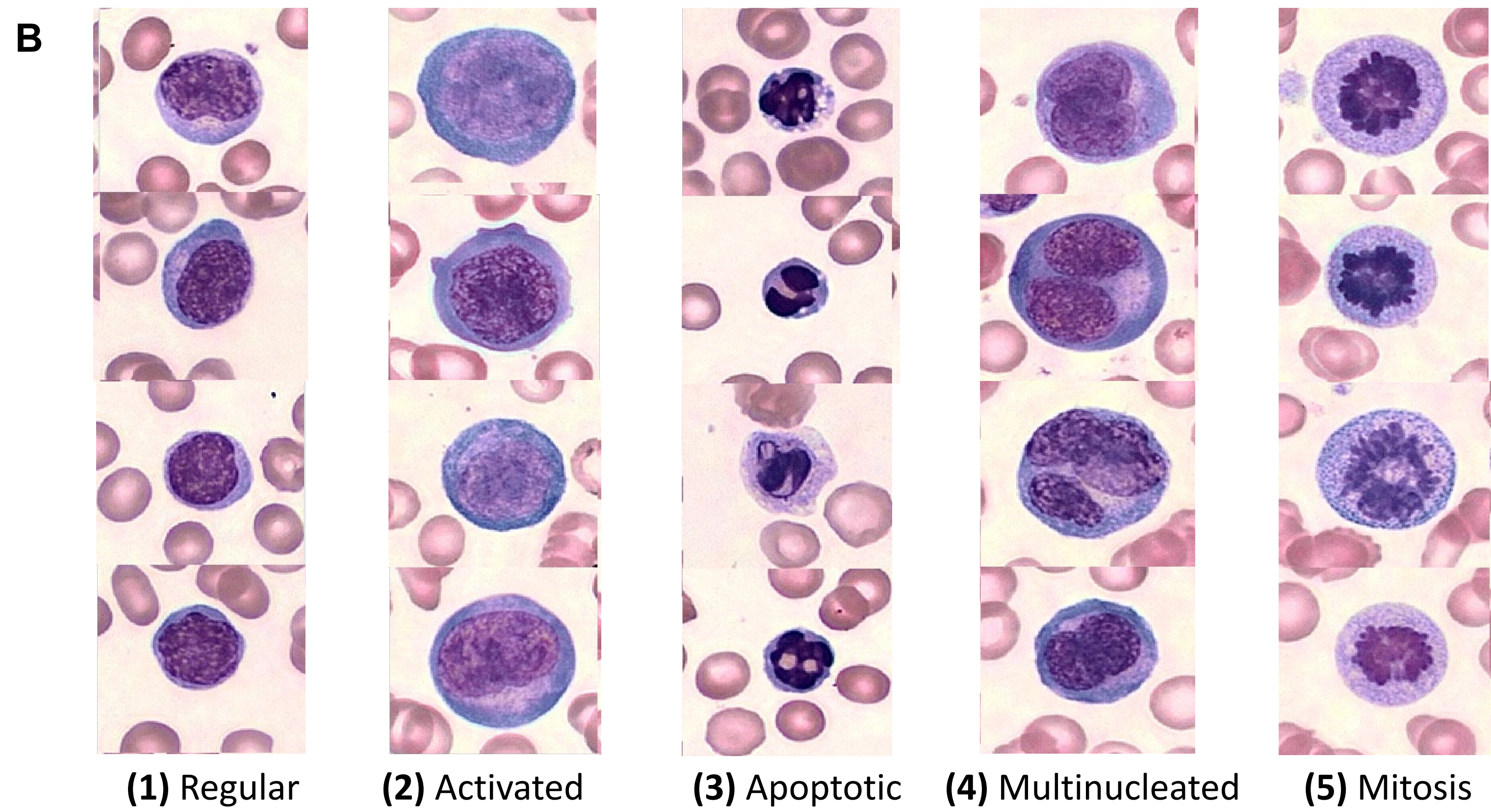
Morphological library of CAR-T cells was established from cells obtained directly from the CAR-T transfusion-bag (Figure A). FFM analysis of consecutive PBS samples obtained from 26 R/R DLBCL patients, treated either with tisagenlecleucel (tisa-cel) or axicabtagene ciloleucel (axi-cel) at the Tel Aviv Sourasky Medical Center between October 2019 - October 2020 was performed.



FFM can retrieve high number of cells even from leukopenic samples (Figure A). A large area of the PBS is scanned (1), following individual WBC automatic detection throughout the scan (2). Each square represents a 100X magnification single Field of View (FOV) (3). Classification of cells in each PBS is determined automatically by the platform software. As CAR-T morphology was in fact described here first, classification was manually reviewed in a cell by cell manner (4).

RESULTS

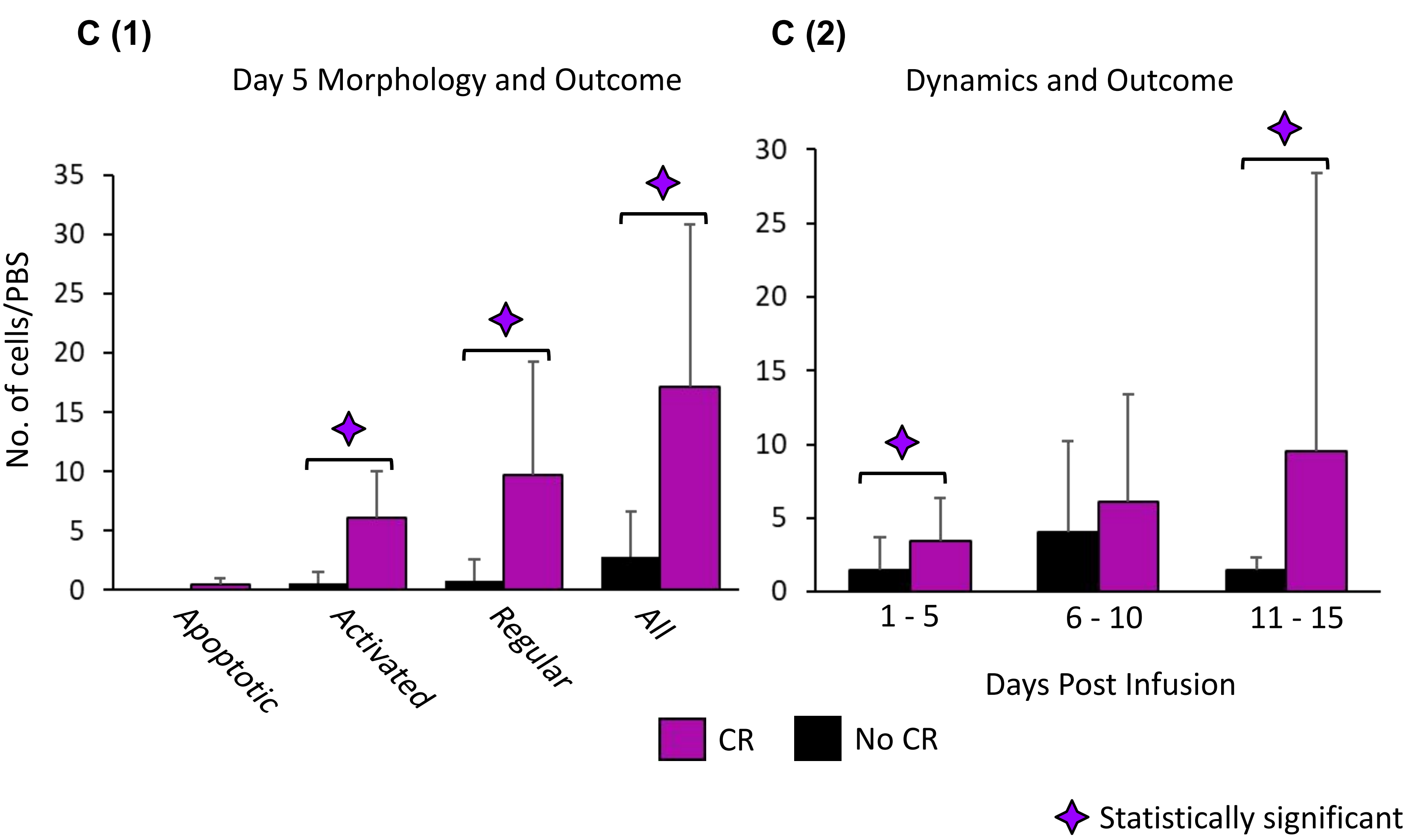
We identified in the CAR-T cell transfusion bags 5 distinct CAR-T morphological sub groups prior to transfusion: regular, activated, apoptotic, multinucleated and cells in mitosis (Figure B). (1) Regular morphology CAR-T cells - small lymphocytes with basophilic cytoplasm (2) Activated CAR-T, large cells with abundant basophilic cytoplasm and reticulated nucleus, with or without nucleoli (characteristics which are typical for reactive lymphocytes) (3) Apoptotic cells, small lymphocytes with pycnotic nuclei, often with vacuoles (4) Multinucleated CAR-T cells and (5) Mitotic CAR-T cells, referring to cells in mitosis, mostly in anaphase.



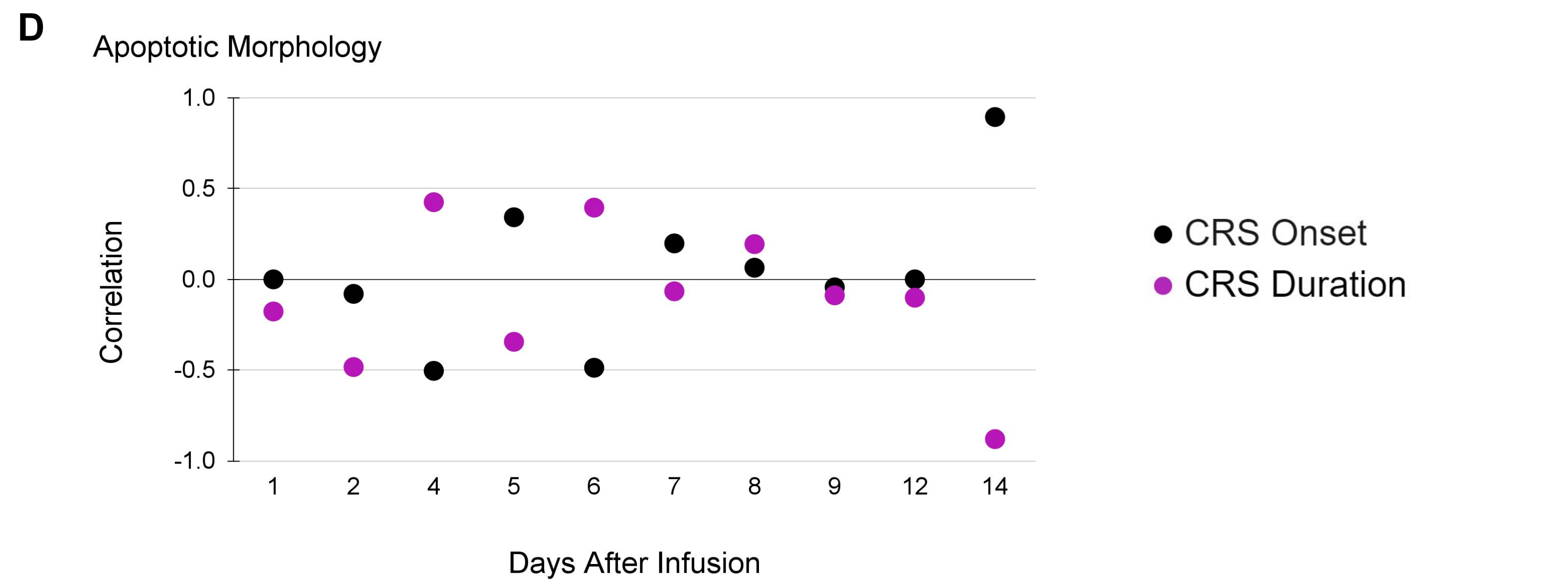
79 PBSs, obtained from 18 patients, were examined with the FFM platform as well as the CellaVision DM1200 system in order to compare current digital technologies. Overall, 24,247 WBC were detected by the FFM platform (average 303 cells/PBS), compared with 4,335 detected by CellaVision system (average 54.4 cells/PBS). Detection of CAR-T was superior with the FFM platform (median difference per PBS - 2, IQR 0-6, $p < 0.001$).

166 PBSs, collected from 26 patients on days 1-15 following CAR-T transfusion, were analyzed by the FFM platform. The average number of total CAR-T cells, measured on Day 5 post transfusion, was significantly ($p = 0.018$) higher in patients who obtained CR at day 30 post CAR-T, reaching 17.1 ± 13.7 cells/PBS vs 2.8 ± 3.4 cells/PBS for patients that not reach CR. Day 5 regular and activated CAR-T cells were particularly higher in patients attaining CR: 9.7 ± 9.6 cells/PBS vs 6.1 ± 3.8 cells/PBS, $p = 0.023$; and 0.8 ± 1.8 cells/PBS vs 0.6 ± 0.9 cells/PBS, $p = 0.005$, respectively (Figure C1).

Dynamics of CAR-T cells in PBS, assessed by measuring average number of activated CAR-T cells on D1-5 and D11-15, was also associated with increased CR rates; average numbers of activated morphology CAR-T were 3.4 ± 2.9 cells/PB and 9.5 ± 18.9 cells/PBS in patients with CR, vs 2.2 ± 0.9 cells/PBS and 1.5 ± 0.9 cells/PBS in patients that did not reach CR, $p = 0.03$ and $p = 0.04$ respectively (Figure C2).



Longer cytokine release storm (CRS) duration was associated with higher number of CAR-Ts with activated morphology, and a lower presence of apoptotic cells on day 14. Higher number of apoptotic cells on day 14 was associated with deferred CRS onset, while lower number of activated morphology cells on day 14 was associated with earlier CRS onset (Figure D)



CONCLUSION

In this study we show that CAR-T cells has heterogeneous morphology identified in PBS. Specific morphological features in specific days were associated with outcome and therapy related toxicities, emphasizing that CAR-T monitoring require quantitative, qualitative and repeated measurements. Thus, morphological CAR-T surveillance using FFM might serve as an available, simple and inexpensive method to provide clinically relevant insights.

CONTACT INFORMATION

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