Hautklinik, Johannes Wesling Klinikum, Minden, Germany

Quantitative IHC analyses for CD30+ cutaneous T-cell lymphoma: chances and challenges

Cassandra Cieslak[1], Christina Mitteldorf [2], Tanja Krömer-Olbrisch[1], Werner Kempf[3], Rudolf Stadler[1]

[1] University Clinic for Dermatology, Johannes Wesling Medical Centre, UKRUB, University of Bochum, Minden, Germany.

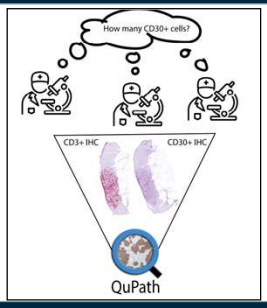
[2] Department of Dermatology, Venereology and Allergology, University Medical Center Göttingen, Göttingen, Germany.

[3] Kempf & Pfaltz, Histologische Diagnostik, Seminarstrasse 1, 8057 Zürich, Zurich, Switzerland; Department of Dermatology, University Hospital Zurich, Gloriastrassse 31, 8091 Zürich, Switzerland.

Project - Introduction

Cutaneous T-cell lymphoma (CTCL) is a malignant manifestation in the skin. The most popular subtype is Mycosis fungoides (MF). The straight focus on the molecular background of the malignancy in the last decades lead into new therapeutic possibilities. The subtype CD30+ MF can be nowadays treated with the CD30 antibody conjugate brentuximab vedotin.

Diagnostic methods are based on Immunohistochemistry (IHC) followed by manual assessment from pathologists or specialized histologists. Since the manual estimated proportion of CD30+ T cells in the skin is always a subjective calculation, there is a need for objective approaches. QuPath, an open-source software for digital pathology image analysis can meet this need.



Materials and Methods

10 samples of MF patients with CD30 expression in different stages were stained for CD3 and CD30 by immunohistochemistry. All slides were scanned using Sysmex Pannoramic DESK Scanner 20x.

A quantitative analysis was performed using QuPath, an open-source software. Simultaneously, each slide was assessed independently by three board certified dermatopathologists. The CD30+/CD3+ positive rate was determined with each of the two methods.

Results

The individual estimates for CD30+/CD3+ cells varied between individual histologists (mean coefficient of variation 0.46; range 0-0.78).

QuPath analysis shows excellent separation between the positive stained cells for CD3 and CD30 IHC and other cells and tissue structures.

The QuPath etimates for CD30+/CD3+ ratios correlate strongly with the mean estimate of the three histologists (Pearson-R 0.93).

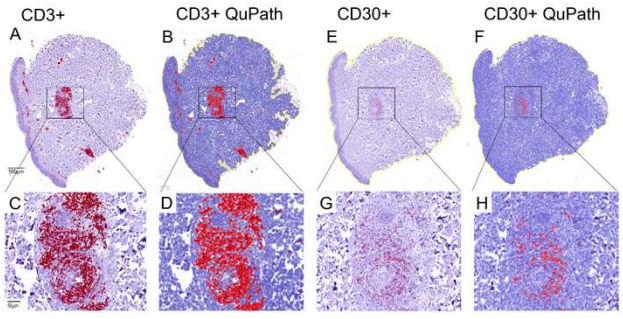


Figure 2: Sample no.6. An example for parallel slides of the IHC-stains CD3+ and CD30+. The CD3+ stained slide (A) is shown with the related QuPath analysis of the slide (B). For better comprehensibility of the analysis results, a 50µm zoom is shown in each case (C, D). The CD30+ IHC stained slide (E), also shown in the QuPath analysis (F) including 50µm Zoom for each (G, H) is shown for a parallel slide. The Histologists mean value of the CD30/CD3 ratio here is 9.2 % and the QuPath value 20.7 %.

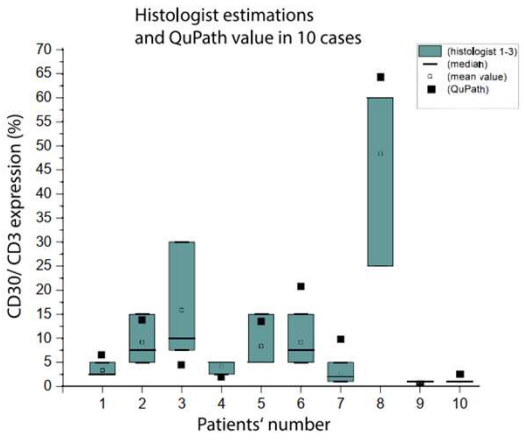


Figure 3: CD30/ CD3 estimations in percent of 3 histologists (green box) including mean value and median value compared to the QuPath value (black box).

Discussion and Conclusion

The good results of the QuPath analysis can be regarded as an independent method for non-subjected slide analysis.

In practice, challenges of this method is to standardize the whole procedure. It has been shown that the interobserver variability evaluating of immunohistochemical markers is high. Therefore, quantitative image analysis, such as QuPath, offers significant advantage and make results better comparable. This is not only relevant for clinical routine, but expecially critical in therapeutic studies addressing targeted molecules.

contact:
Cassandra.Cieslak@rub.de