Assessment Run 10 2004

CD15

The slide to be stained for CD15 comprised:
1. Lymph node with Hodgkin's lymphoma, mixed cellularity (MC), 2. Lymph node with Hodgkin's lymphoma, nodular sclerosing (NS), 3. Spleen with acute myeloid leukaemia (AML), 4. Peritoneal malignant mesothelioma, 5. Lung adenocarcinoma, 6. Tonsil.

Criteria for assessing a CD15 staining as optimal included:
A strong and distinct predominantly membranous staining and reaction of the Golgi apparatus of the Reed-Sternberg and Hodgkin's cells in both cases of Hodgkin's lymphoma, a membranous and cytoplasmic staining of the neoplastic cells of the AML, and a focal cytoplasmic reaction of the neoplastic cells of the lung carcinoma, whereas the malignant mesothelioma should be negative. A dot-like nuclear staining in neutrophils and the adenocarcinoma was accepted (see Fig. 3).

71 laboratories submitted stainings. At the assessment 23 achieved optimal staining (32 %), 13 good (18 %), 11 borderline (16 %) and 24 poor staining (34%).

The following mAbs were used:
clone C3D-1 (DakoCytomation, n=45)
clone MMA (Becton Dickinson, n=16; Cell Margue, n=1; NeoMarkers, n=1)
clone BY87 (Ventana, n=3; Novocastra, n=2)
clone H198 (Becton Dickinson, n=2)
clone Tu9 (Quartett Immunodiagnostika, n=1)

In this assessment optimal stainings could be obtained with the mAbs MMA (15/18), C3D-1 (7/45) and Tu9 (1/1). In the optimal protocols all used HIER, predominantly (22/23) with Tris-EDTA/EGTA pH 9 as the heating buffer. MMA was used in the range of 1:10 – 1:40 and C3D-1 in 1:10 – 1:25, depending on the total sensitivity of the used protocol. Tu9 was used in 1:3.

The majority of laboratories were able to detect CD15 in the neoplastic cells of the AML, whereas the demonstration of CD15 in the two Hodgkin's diseases only was achieved with an optimal protocol.

The most frequent causes of insufficient stainings (often in combination) were:
- Inappropriate choice of primary Ab
- No epitope retrieval or proteolytic pretreatment
- Insufficient HIER: Too short heating time often in combination with citrate pH 6 as the heating buffer
- Too low concentration of the primary antibody.

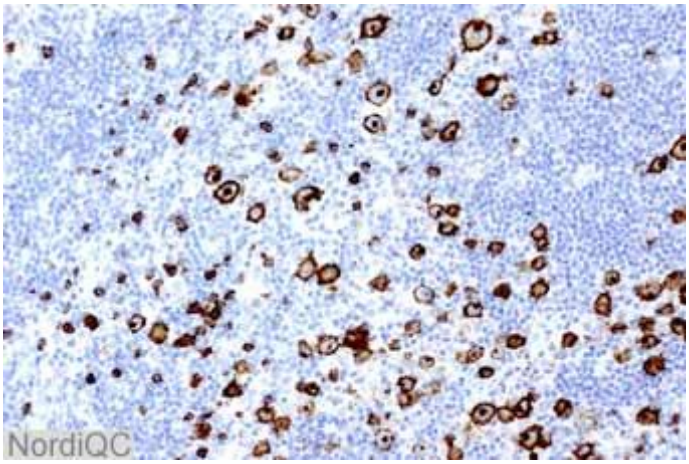

Fig. 1a
Optimal CD15 staining (mAb clone MMA) of the neoplastic cells in the lymph node with Hodgkin's disease (MC). The Reed-Sternberg and Hodgkin's cells show a strong membranous staining and a dot-like positivity.

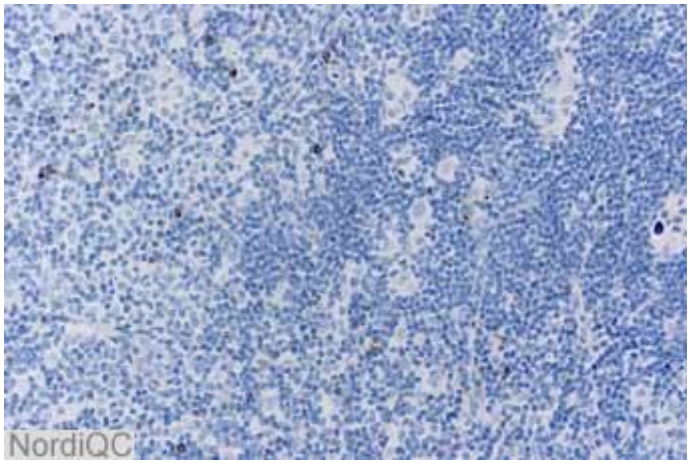

Fig. 2a
Insufficient CD15 staining of the neoplastic cells in the lymph node with Hodgkin's disease (MC). The Reed-Sternberg and Hodgkin's cells are negative. Only the neutrophil granulocytes are stained.

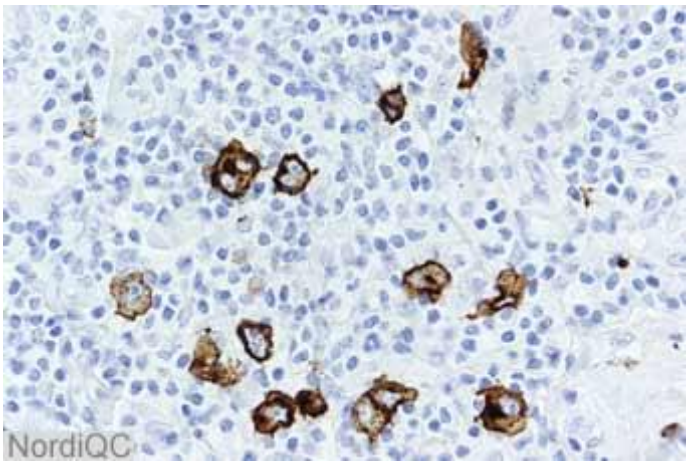

Fig. 1b
Optimal CD15 staining (mAb clone C3D-1) of the neoplastic cells in the lymph node with Hodgkin's disease (NS). The Reed-Sternberg and Hodgkin's cells show a strong membranous staining and focally a dot-like positivity.

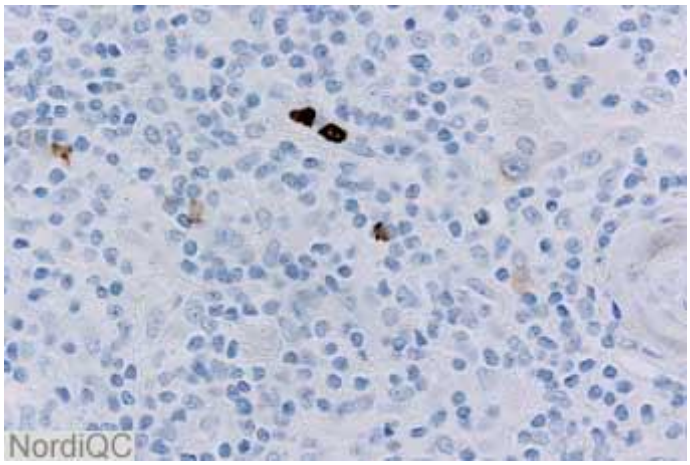

Fig. 2b
Insufficient CD15 staining of the neoplastic cells in the lymph node with Hodgkin's disease (NS). The Reed-Sternberg and Hodgkin's cells are only weakly stained. Only the neutrophil granulocytes are strongly stained.

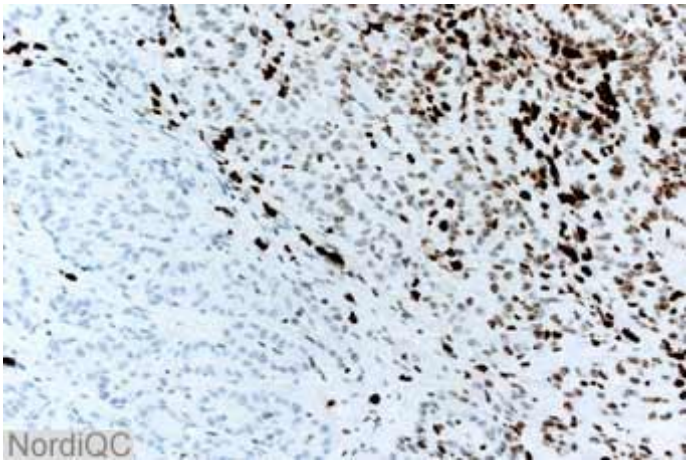

Fig. 3
Frequently a positive nuclear reaction for CD15 is seen in areas with cells revealing a high CD15 expression (e.g. necrosis) primarily observed in high sensitive prototols using HIER in Tris-EDTA/EGTA pH 9. However this high sensitivity is often needed for the demonstration of the relatively low CD15 expression in Reed-Sternberg and Hodgkin's cells.

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