GRADING OF POSTCAPILLARY VENULES IN LYMPH NODES: OBSERVER
VARIATION AND REPRODUCIBILITY IN SUBJECTIVE ESTIMATION AND
MORPHOMETRIC MEASUREMENTS

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ABSTRACT

We wanted to estimate variation and reproducibility in
grading and in morphometric measurements of postcapillary
venules. Five pathologists measured 100 postcapillary venules
from 20 axillary lymph nodes by subjective and morphometric
means. Range of variation was similar in subjective and
morphometric measurements. The correlation coefficients
between subjective and morphometric measurements varied from
0.62 to 0.93. On subjective grading of the individual post-
capillary venules the kappa coefficient was 0.50 (90% limits:
0.40-0.56). On morphometry ICC was 0.74 (0.70-0.78). On
morphometric measurements of individual lymph nodes ICC was
0.82. The results show that grading and morphometric measure-
ments are very similar in respect to variability and repro-
ducibility in this kind of experiment.

INTRODUCTION

Morphological variation in the structure of postcapillary
venules (PCVs) is known to reflect the activity of local immune
response (Kotani et al., 1980, Kittas and Henry 1981, Syrjänen
1982). In human cancer draining lymph nodes high PCV endo-
thelium seems to be a favourable prognostic sign (Syrjänen
1982). So far only subjective grading methods for PCVs have
been used (Kotani et al., 1980, Kittas and Henry 1981, Syrjä-
nen 1982). In the present communication we wanted to compare
subjective grading and morphometric measurements of PCV para-
meters.

MATERIALS AND METHODS

A total of 20 paraffin embedded axillary lymph nodes of breast cancer patients were studied. From 5 μm thick sections 5 pathologists made their subjective estimations of 5 selected PCVs at a magnification of 63 (objective lens) x 10 (eye piece). For morphometric measurements of PCVs eye-piece graticule (E1, Graticules Ltd., England) was used at the same magnification. The subjective grading (three grades) performed as described earlier (Syrjänen 1982). On morphometry the following dimensions were measured: Dpcv: the diameter of the PCV; Dlu: the lumen of the PCV; Hend: the height of the endothelium (Syrjänen and Naukkarinen 1981).

After measurements the following parameters were calculated: (1) The mean grade estimate, the mean of five grade estimates of the same vessel, grading performed by 5 observers. (2) The grade index, the mean of grades of five vessels of the same lymph node as graded by a single observer. (3) The mean grade index, the mean of five grade indexes, grading performed by 5 observers. (4) The mean height estimate, the mean of five Hend values of the same vessel, measured by 5 observers. (5) The height index, the mean of Hend values of five vessels of the same lymph node measured by a single observer. (6) The mean height index, the mean of five height indexes (five observers) of the same node. The standard deviations and the coefficients of variation (CV) of the above parameters were calculated. Pearson's r correlation coefficient was calculated for the estimates and indexes (Table 1). The interobserver reproducibility was estimated with the kappa coefficient (Landis and Koch 1977, Kraemer 1980, Selkäinaho 1983) and the intraclass correlation coefficient (ICC) (Cochran 1968, Selkäinaho 1983).

RESULTS

Coefficients of variation of grading and morphometric measurements of the individual PCVs varied from 0% to 39% on subjective grading, and from 3% to 44% on morphometry. CVs of the mean grade indexes and the mean height indexes varied from 0% to 20% (mean grade index) and from 2% to 21% (mean height index). Pearson's r correlation coefficients for the results on individual postcapillary venules and individual lymph nodes are shown in Table 1.

Table 1. Pearson's r correlation coefficients between differ-
ent indices of PCVs. (90% confidence limits shown in brackets).
For definitions see the text.

Correlated parameters	Individual observers	Pooled results
Grade estimate/ Height estimate	100 estimates of PCVs: I 0.79 (0.72-0.85) II 0.89 (0.85-0.92) III 0.62 (0.50-0.71) IV 0.72 (0.63-0.79) V 0.74 (0.66-0.81)	500 estimates of PCVs: 0.74 (0.70-0.77)
Mean grade estimate/ Mean height estimate	100 mean estimates of PCVs: 0.88 (0.84-0.91)	
Grade index/ Height index	20 indexes: I 0.87 (0.73-0.94) II 0.91 (0.81-0.96) III 0.65 (0.36-0.83) IV 0.80 (0.60-0.90) V 0.93 (0.86-0.97)	100 indexes: 0.79 (0.72-0.85)
Mean grade index/ Mean height index	20 mean indexes: 0.90 (0.80-0.96)	

On subjective grading of PCVs κ was 0.50 (90% limits:
0.43-0.56) and on morphometric measurements of PCVs ICC
was 0.74 (0.70-0.78). On morphometric analysis of the indi-
vidual lymph nodes (height index) ICC was 0.82 (0.70-0.89).

DISCUSSION

The range of variation was of the same degree in both
approaches and this applied for individual venules and indi-
vidual indices. The reproducibility of the results was also
similar. However, conclusions should be careful because ICC
is not the method of choice for discrete variables. The
results corresponded to moderate (grade estimate, height
estimate) and to substantial (height index) degree of repro-
ducibility. The results suggest that both of these methods

under conditions of the experiment can be applied safely. However, the potential bias must be taken into consideration. That bias may play a part is shown by the correlation coefficient values of different observers. They showed considerable variation. Low correlation coefficients suggest that grading is biased or badly reproducible or that morphometry is characterized by these features. The external reference offered by morphometry will reduce bias. However, human factors in measurement are apt to decrease reproducibility also in morphometry.

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