

WHICH VALUE CAN BE USED FOR LUC AS A POSITIVITY THRESHOLD FOR MAKING SLIDES?

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INTRODUCTION

The Advia 2120i system uses peroxidase staining for white blood cells differential testing. So white blood cells are grouped into 6 populations, such as neutrophil-, eosinophil-, basophil granulocytes, monocytes, lymphocytes and the additional cell group is large unstained cells (LUC) is differentiated (Figure 1). Peroxidase-negative cells, large lymphocytes, plasma cells, blasts are counted to large unstained cells (LUC) category. The manufacturer suggests normal range for LUC < 4%. If the sample LUC > 4%, they suggest to make a slide and analyze the morphology of cells by microscopy. We observed that a lot of "unnecessary" smear evaluation were made based on the results of LUC. When LUC is 5-6% only a few (1-6) reactive/granulated lymphocytes were found, generally. The aim of this study was to find a reliable positivity threshold for LUC, where the results by microscopy give additional information to the clinician beyond the mere numerical data.

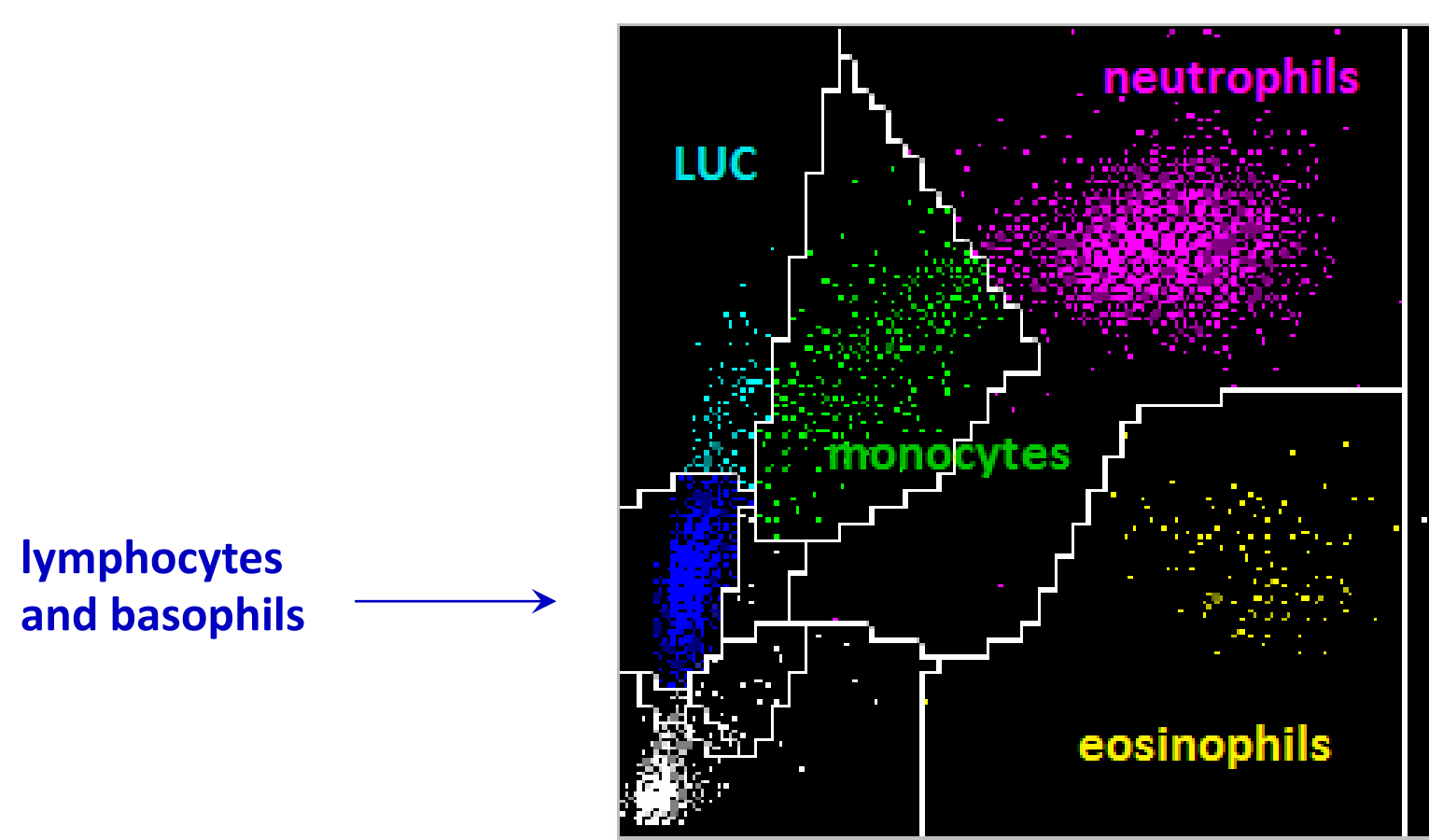


Figure 1. Peroxidase cytogram (number of basophils comes from baso cytogram /not shown/)

MATERIALS AND METHODS

The ROC curve analysis was used to obtain the appropriate results. First of all, it was clarified which patients results will be analysed (e.g. myeloperoxidase deficiency were excluded from the examination). Blood samples of 350 adults and 145 children were analysed by automated Advia 2120i and microscopy.

Three analyses were performed:

Analysis "1": it was considered to be positive the results of patients when blast cells or reactive/granulated lymphocytes (irrespective of their number) were found by microscopy.

Analysis "2": positive results were if blast cells or a few (4-6) or more reactive/granulated lymphocytes were found.

Analysis "3": positive results were if blast cells or several (>7) reactive/granulated lymphocytes were found.

In all three cases, ROC curve analyses were performed to find the best sensitivity and specificity rates to get the appropriate positivity threshold value for LUC.

		test outcome		
		negative (-)	positive (+)	
real condition	healthy (-)	true negative (TN)	false positive (FP)	→ Specificity TN/(TN + FP)
	ill (+)	false negative (FN)	true positive (TP)	→ Sensitivity TP/(FN + TP)

Table 1. Sensitivity and specificity.

Sensitivity and specificity reflect the diagnostical utility of a laboratory parameter (Table 1).

Sensitivity measures the proportion of actual positives which are correctly identified as such (e.g. the percentage of sick people who are correctly identified as having the condition).

$$\text{sensitivity} = TP / (FN + TP)$$

Specificity measures the proportion of negatives which are correctly identified (e.g. the percentage of healthy people who are correctly identified as not having the condition).

$$\text{specificity} = TN / (TN + FP)$$

SUMMARY

Results were summarized in Table 2. Although the highest collective sensitivity and specificity were found at LUC > 4%, there is a high false positive rate. However, in the analysis „3” there is a quite sensitivity and specificity, and the false positive rate is rather low. So we use positivity threshold value for LUC > 6% in our practice.

RESULTS

Analysis "1": it was considered to be positive the results of patients when blast cells or reactive/granulated lymphocytes (irrespective of their number) were found by microscopy. Number of positive case (=1) were 260 and negative case (=0) were 235. The positivity threshold by ROC analysis were found LUC > 4%. It is in agreement with the recommendation of the manufacturer. In this case, the true negative rate (TNR; specificity) was 74.76% and true positive rate (TPR, sensitivity) was 88.81%. High number of false positive results (FPR) were found: 25.24%. Area under the ROC curve (AUC) was 0.84 (Figure 2).

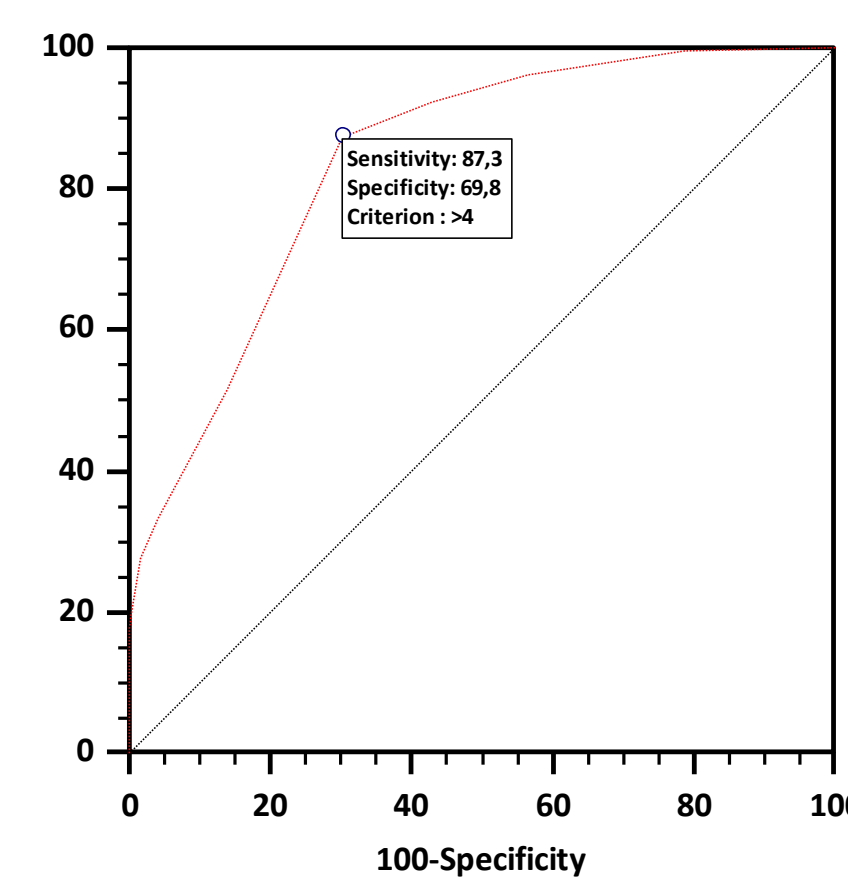


Figure 2. ROC curve and data of patients according to the analysis „1”.

LUC	Sensitivity	95% CI	Specificity	95% CI
71	100.00	98.6 – 100.0	0.00	0.0 – 1.6
>1	99.62	97.9 – 100.0	21.28	16.2 – 27.1
>2	96.15	93.0 – 98.1	43.40	37.0 – 50.0
>3	92.31	88.4 – 95.2	57.02	50.4 – 63.4
>4	87.31	82.6 – 91.1	69.79	63.5 – 75.6
>5	51.54	45.3 – 57.8	85.96	80.8 – 90.1
>6	33.46	27.8 – 39.6	95.74	92.3 – 97.9
>7	27.69	22.3 – 33.6	98.30	95.7 – 99.5
>8	19.62	15.0 – 25.0	99.57	97.7 – 100.0
>9	17.69	13.3 – 22.9	100.00	98.4 – 100.0
>10	14.23	10.2 – 19.1	100.00	98.4 – 100.0
>11	10.38	7.0 – 14.7	100.00	98.4 – 100.0
>12	6.54	3.9 – 10.3	100.00	98.4 – 100.0
>13	5.00	2.7 – 8.4	100.00	98.4 – 100.0
>14	2.69	1.1 – 5.5	100.00	98.4 – 100.0
>15	2.31	0.9 – 5.0	100.00	98.4 – 100.0
>16	1.54	0.4 – 3.9	100.00	98.4 – 100.0
>19	1.15	0.2 – 3.3	100.00	98.4 – 100.0
>21	0.77	0.09 – 2.8	100.00	98.4 – 100.0
>24	0.38	0.010 – 2.1	100.00	98.4 – 100.0
>29	0.00	0.0 – 1.4	100.00	98.4 – 100.0

Analysis "2": the results were considered to be positive, if blast cells or a few (4-6) or more reactive/granulated lymphocytes were found by microscopy. In this case number of positive results were 174 and negative were 321. The positivity threshold was LUC > 5%. TNR was 83.27%, TPR was 66.25% and FPR was 16.73%. Area under the ROC curve (AUC) was 0.81 (Figure 3).

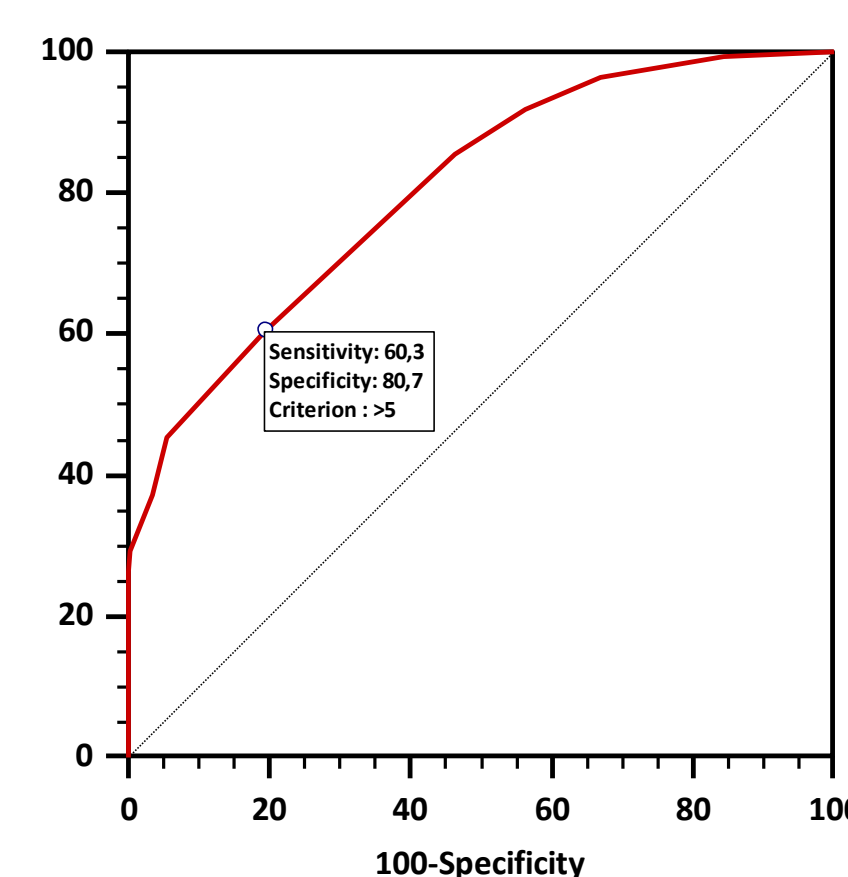


Figure 3. ROC curve and data of patients according to the analysis „2”.

LUC	Sensitivity	95% CI	Specificity	95% CI
71	100.00	97.9 – 100.0	0.00	0.0 – 1.1
>1	99.43	96.8 – 100.0	15.58	11.8 – 20.0
>2	96.55	92.6 – 98.7	33.02	27.9 – 38.5
>3	91.95	86.9 – 95.5	43.61	38.1 – 49.2
>4	85.63	79.5 – 90.5	53.58	48.0 – 59.1
>5	60.34	52.7 – 67.7	80.69	75.9 – 84.9
>6	45.40	37.9 – 53.1	94.39	91.3 – 96.6
>7	37.36	30.2 – 45.0	96.57	94.0 – 98.3
>8	29.31	22.7 – 36.7	99.69	98.3 – 100.0
>9	26.44	20.1 – 33.6	100.00	98.9 – 100.0
>10	21.26	15.4 – 28.1	100.00	98.9 – 100.0
>11	15.52	10.5 – 21.8	100.00	98.9 – 100.0
>12	9.77	5.8 – 15.2	100.00	98.9 – 100.0
>13	7.47	4.0 – 12.4	100.00	98.9 – 100.0
>14	4.02	1.6 – 8.1	100.00	98.9 – 100.0
>15	3.45	1.3 – 7.4	100.00	98.9 – 100.0
>16	2.30	0.6 – 5.8	100.00	98.9 – 100.0
>19	1.72	0.4 – 5.0	100.00	98.9 – 100.0
>21	1.15	0.1 – 4.1	100.00	98.9 – 100.0
>24	0.57	0.01 – 3.2	100.00	98.9 – 100.0
>29	0.00	0.0 – 2.1	100.00	98.9 – 100.0

Analysis "3": only those results were positive when blast cells or several (>7) reactive/granulated lymphocytes were found by microscopy. Number of positive results were 130 and negative 365. The positivity threshold was LUC > 6%. TNR: 94.08%, TPR: 56.45% and FPR: 5.92%. Area under the ROC curve (AUC) was 0.82 (Figure 4).

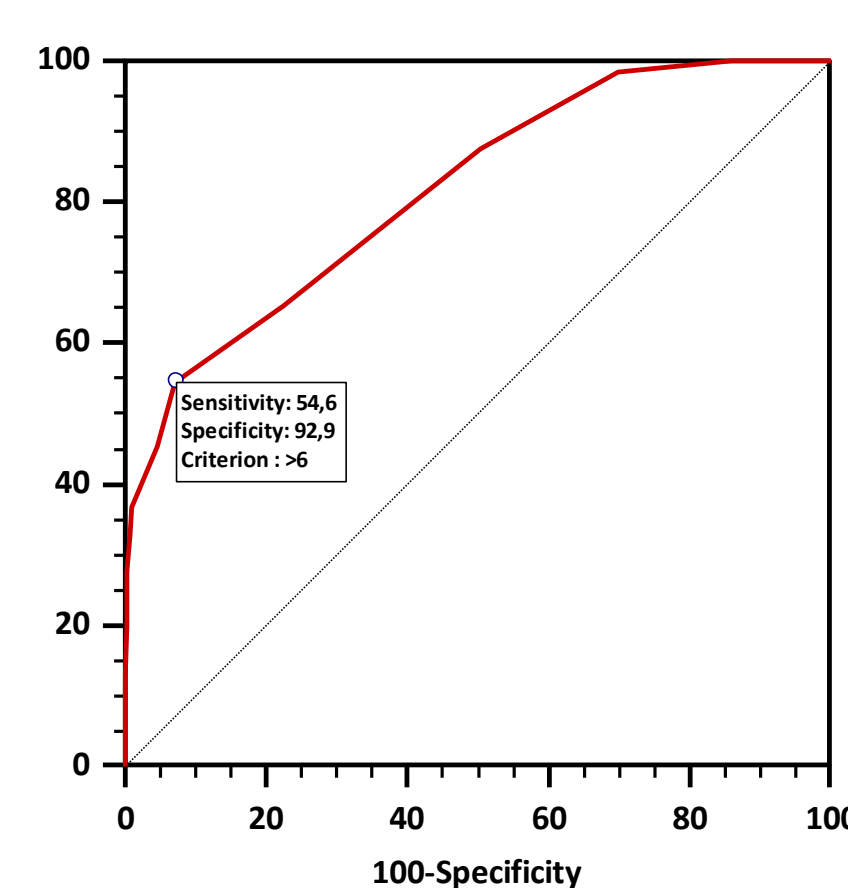


Figure 4. ROC curve and data of patients according to the analysis „3”.

LUC	Sensitivity	95% CI	Specificity	95% CI
71	100.00	97.2 – 100.0	0.00	0.0 – 1.0
>1	100.00	97.2 – 100.0	13.97	10.6 – 18.0
>2	98.46	94.6 – 99.8	30.14	25.5 – 35.1
>3	93.08	87.3 – 96.8	39.73	34.7 – 44.9
>4	87.69	80.8 – 92.8	49.59	44.3 – 54.8
>5	65.38	56.5 – 73.5	77.53	72.9 – 81.7
>6	54.62	45.7 – 63.4	92.88	89.7 – 95.3
>7	45.38	36.6 – 54.3	95.34	92.6 – 97.3
>8	36.92	28.6 – 45.8	98.90	97.2 – 99.7
>9	33.08	25.1 – 41.9	99.18	97.6 – 99.8
>10	27.69	20.2 – 36.2	99.73	98.5 – 100.0
>11	20.00	13.5 – 27.9	99.73	98.5 – 100.0
>12	13.08	7.8 – 20.1	100.00	99.0 – 100.0
>13	10.00	5.4 – 16.5	100.00	99.0 – 100.0
>14	5.38	2.2 – 10.8	100.00	99.0 – 100.0
>15	4.62	1.7 – 9.8	100.00	99.0 – 100.0
>16	3.08	0.8 – 7.7	100.00	99.0 – 100.0
>19	2.31	0.5 – 6.6	100.00	99.0 – 100.0
>21	1.54	0.2 – 5.4	100.00	99.0 – 100.0
>24	0.77	0.02 – 4.2	100.00	99.0 – 100.0
>29	0.00	0.0 – 2.8	100.00	99.0 – 100.0

analysis	threshold for LUC	sensitivity	specificity	false positive rate
analysis „1”	> 4%	87.3%	69.8%	30.2%
analysis „2”	> 5%	60.3%	80.7%	19.3%
analysis „3”	> 6%	54.6%	92.9%	7.1%

Table 2. Summary of results.