

## Leukocyte Drumsticks in Chronic Granulocytic Leukemia and Related Disorders

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**I**N PREVIOUS REPORTS we demonstrated that leukocyte alkaline phosphatase and catalase concentrations were altered in leukemia.<sup>9,21</sup> In chronic granulocytic leukemia (CGL), leukocyte alkaline phosphatase is low and catalase is high. These abnormal values return to normal in most patients with CGL in response to treatment. These enzyme changes were noted in the early stages of this disease at times when there were very few immature cells in the peripheral blood. This suggested that there may be two types of neutrophils in CGL, each with similar morphologic but dissimilar biochemical characteristics.

In order to explore further the "double cell" population theory in CGL, the "drumstick" of the polymorphonuclear neutrophils was investigated. Davidson and Smith were the first to identify these peculiar nuclear appendages, and to define their relationship to the sex-chromatin of the cells.<sup>4</sup> Recent studies on the chromosomes of neoplastic cells from mammalian tumors have demonstrated that these cells have altered chromosomal patterns. It was speculated that these chromosomal aberrations might result in changes in the drumstick count of the granulocytes of patients with CGL which could be detected by means of careful morphologic study.

### METHODS AND MATERIALS

Davidson and Smith classified five types of nuclear appendages in mature neutrophils, drumsticks, sessile nodules, small clubs, minor lobes and racket formation.<sup>4</sup> They considered only drumsticks to be related to the sex chromatin. On the other hand, Kosenow believes that sessile nodules are of equal sex-diagnostic significance.<sup>11</sup> In this study, Davidson's classification was adopted. Undoubtedly, some of the drumsticks are hidden or partly hidden by one of the lobes of the nucleus, but we have reckoned as characteristic only those which are discrete.

As a preliminary study, the blood films of 100 normal adult female and 50 normal adult male subjects were examined in order to determine the normal range and the reproducibility of the method. Blood films of good quality were stained in the usual way by the May-Gruenwald-Giemsa technic. One thousand neutrophils were examined and each nuclear appendage was classified into one of the five types described by Davidson. The frequency of each type of appendage was expressed as number per 1000 neutrophils. In addition the frequency of occurrence of the six drumsticks and of the first drumstick in the neutrophils, as described by Davidson and Smith in their original report, also were evaluated. The relationship of the frequency of each nuclear appendage to the lobe counts of mature neutrophils or ages was investigated. The detailed analyses will be reported elsewhere.

Following the examination of normal individuals, the frequencies of drumsticks and the other nuclear appendages were determined in patients with CGL, leukemoid reactions,

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or inflammatory leukocytosis. This included 9 cases of CGL, 1 case of myeloid metaplasia, 6 cases of leukemoid reaction and 10 cases of inflammatory leukocytosis (15,000–25,000 leukocytes/mm.<sup>3</sup>) due to either acute appendicitis or cholecystitis.

## RESULTS

1. *Frequency of drumsticks in normal individuals.* As shown in table 1, there was the highly significant difference between the drumstick counts of the normal male and female subjects and the frequency distribution of the drumstick counts in each sex was separated in contrast to those of the other nuclear appendages, which showed also significant sexual differences comparing mean values. The results of drumstick counts on normal women by several authors are summarized in table 2. The comparison of the frequency distribution of our subjects with that of Davidson's and Briggs' data is shown in figures 1 and 2.

2. *Frequency of drumsticks in female patients with CGL, leukemoid reactions and inflammatory leukocytoses.* The drumstick counts in all patients with CGL in relapse obviously were below the normal range and were significantly lower than the values obtained from patients with the other disorders (table 3). The drumstick counts in CGL increased rapidly during treatment and returned to the normal levels in each of the four cases that went into complete remission (figs. 3 and 4). No change in the drumstick counts in the neutrophils from patients with inflammatory leukocytosis following treatment was noted. This included two cases in which very low values had been recorded prior to therapy. The low values of these two cases probably represents a variation from the normal, rarely found in females, but qualitatively different from the changes of CGL.

Table 1.—*Frequency\* of Each Nuclear Appendage of Neutrophils and Sex Difference in Normal Individuals*

Type of appendage	Female (100 cases)				Male (50 cases)				Sex difference (t-test)		
	Min.	Max.	Mean*	S.D.†	Min.	Max.	Mean†	S.D.	M*—M†	t	p
Drumsticks	8	47	25.15	7.79	0	6	2.78	1.56	+22.37	20.07	0.001
Sessile nodules	93	318	184.52	51.02	37	169	80.59	29.46	+103.93	13.32	0.001
Small clubs	18	170	63.33	21.88	57	232	124.28	32.27	-60.95	13.60	0.001
Minor lobes	0	18	5.62	3.07	0	10	3.98	2.77	+1.64	3.19	0.01

\*The frequency is expressed as number per 1000 neutrophils.

†Standard Deviation.

Table 2.—*Frequency of Drumstick in Normal Female Subjects—Summary of Published Data*

Reference	No. of cases	First Drumstick*			Six Drumsticks†			Per 10 <sup>3</sup> Neutrophils		
		Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean
Davidson	125	1	250	36	36	588	277	10	166	26.3
Koenow	100			37						30.4
Lueer	24									51.4
Briggs	100							2	58	19.6
Maruyama	31	2	148		33	600				13.3
Ito	50									24.7
Ooga	115									24.6
Authors	100	1	133	30	47	586	249	8	47	25.2

\*The frequency of the first drumstick found in the neutrophils.

†The frequency of the six drumsticks in the neutrophils.

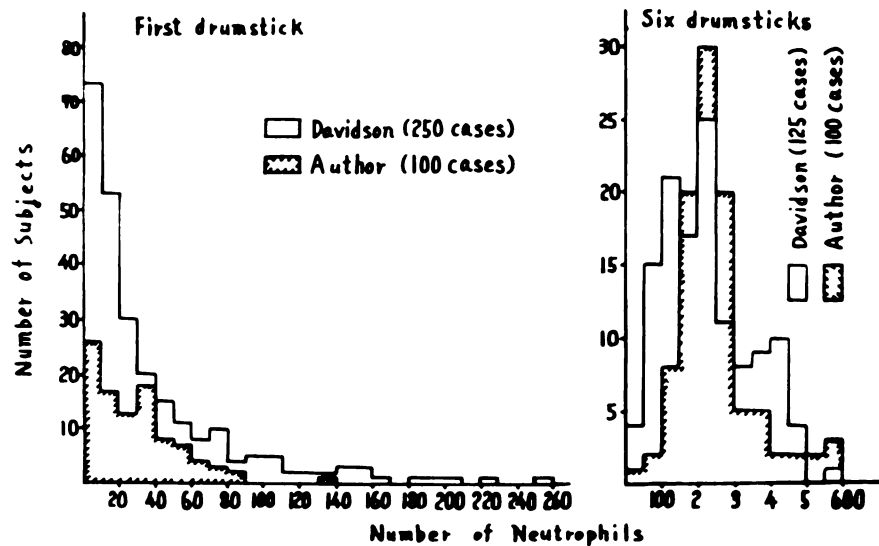


Fig. 1.—The graph on the left shows the frequency distribution of the first drumstick found in the neutrophils and the graph on the right shows that of the six drumsticks in the neutrophils in normal female blood films.

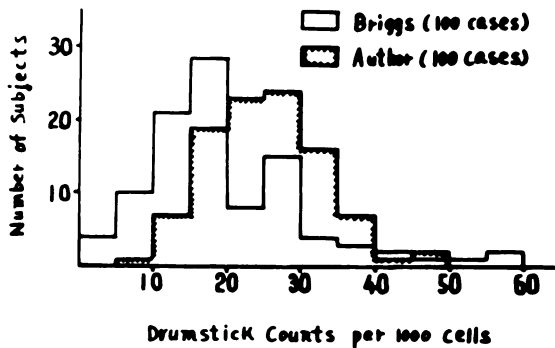


Fig. 2.—The frequency distribution of drumstick counts in 1000 neutrophils in normal female subjects.

In patients with CGL there was close correlation between the return to normal of drumstick counts and the leukocyte alkaline phosphatase and catalase concentrations following therapy. A typical example of these relationships is shown in figure 5. In this group of patients there were two with atypical clinical courses. The first patient initially was thought to have CGL. He had a relative low leukocyte count, a huge spleen, numerous erythroblasts in the peripheral blood, an abnormally high platelet count, and very scanty bone marrow by needle aspiration. These findings indicated that the patient probably had myelosclerosis with myeloid metaplasia rather than CGL. The leukocyte alkaline phosphatase content in this patient was abnormally high prior to any treatment, and remained high during eight months of remission in response to busulfan therapy. Drumstick counts in this patient remained in the normal range throughout his entire clinical course. The second was a patient with typical CGL who initially showed hematologic and clinical im-

**Table 3.—Frequency\* of Each Nuclear Appendage in Chronic Granulocytic Leukemia (CGL), Leukemoid Reactions (LR), Inflammatory Leukocytoses (IL) and Normal Women (N)**

Type of appendage	Diag.	No.	Min.	Max.	Mean	S.D.
Drumsticks	CGL	8	3	8	6.40	2.05
	LR	6	7	34	22.24	8.18
	IL	10	3	27	24.51	12.77
	N	100	8	47	25.15	7.79
Sessile nodules	CGL	8	51	159	97.37	35.24
	LR	6	70	177	113.27	44.39
	IL	10	20	213	141.81	60.36
	N	100	93	318	184.52	51.02
Small clubs	CGL	8	0	52	16.08	17.07
	LR	6	7	70	29.29	25.02
	IL	10	16	94	48.93	27.86
	N	100	18	170	63.33	21.88
Minor lobes	CGL	8	0	6	2.21	2.68
	LR	6	0	11	5.08	4.04
	IL	10	0	11	4.72	3.70
	N	100	0	18	5.62	3.07

\*The frequency is expressed as number per 1000 neutrophils.

provement in response to busulfan therapy associated with concomitant elevation of both the drumstick and leukocyte alkaline phosphatase concentrations. Later acute relapse occurred, at which time leukocyte phosphatase concentration was found to be greatly elevated and the drumstick count became moderately increased. This was in contrast to the low values usually observed in advanced CGL.

In figure 6 the relationship between the drumstick count and the percentage of immature cells in the peripheral blood is shown. There appears to be close negative correlation between them, suggesting that the drumstick count is related to the severity of the disease.

The relationship between drumstick counts and the lobe counts of mature neutrophils is shown in figure 7. Fairly good correlation was observed between the number of drumsticks counted and the number of lobes of the segmented leukocytes both in normal subjects and patients with CGL. This relationship exists in spite of the reduced number of drumsticks found in the cells of patients with CGL. It was of interest that no drumsticks were found in the 3-lobe segmented neutrophils of three cases of CGL. In remission, the drumstick-lobe relationship in CGL is even more consistent with that of normal individuals. No exceptionally high drumstick counts were encountered in the cells from any of the patients in this study. This is in contrast to the leukocyte alkaline phosphatase and catalase levels which often were markedly elevated.

3. *Frequency of nuclear appendages other than drumstick in leukocytes of patients with CGL, leukemoid reactions and inflammatory leukocytoses.* Prior

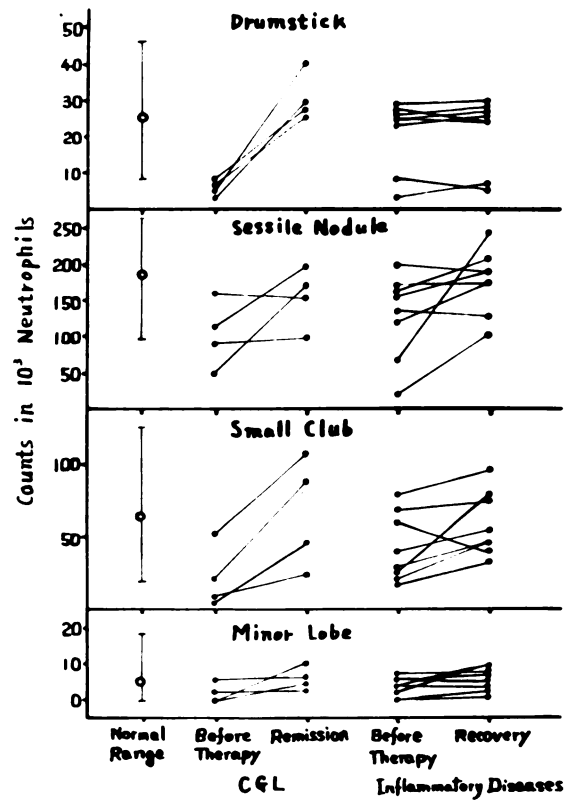


Fig. 3.—The comparison of the frequencies of each nuclear appendage before and after treatment in women with chronic granulocytic leukemia or inflammatory leukocytoses.

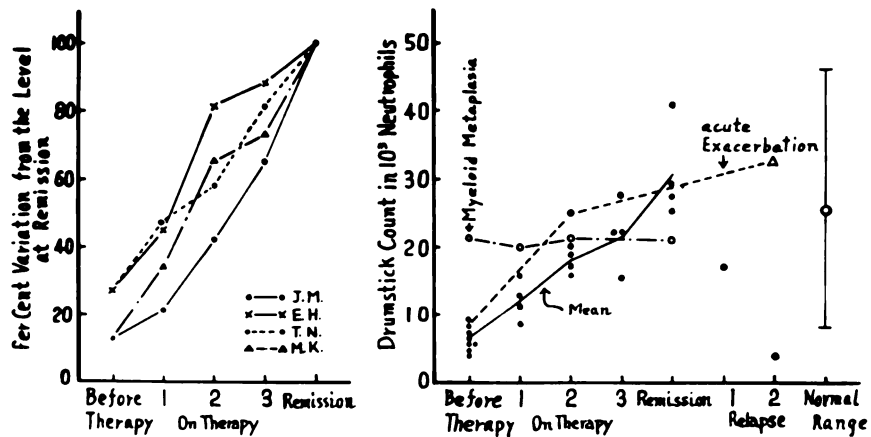


Fig. 4.—The effects of treatment on the drumstick counts of women with chronic granulocytic leukemia.

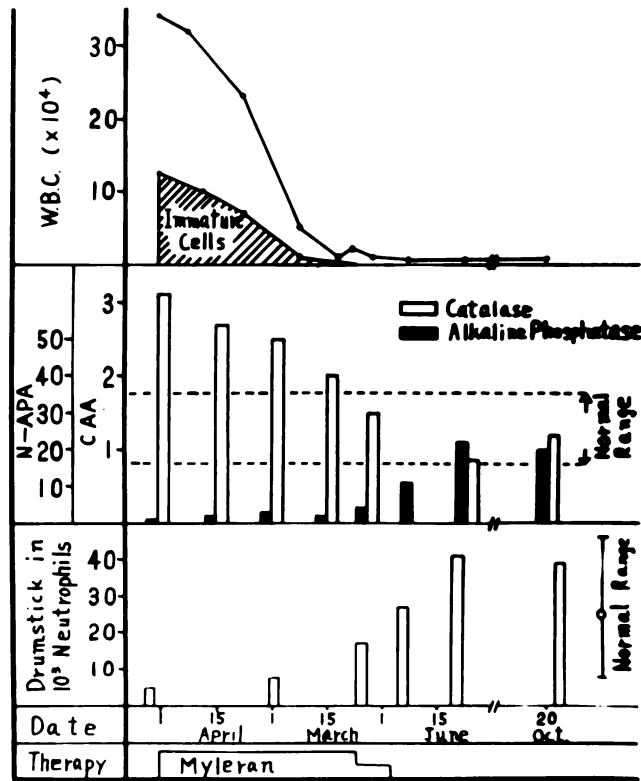


Fig. 5.—The relationship between the return to normal of the drumstick counts and the leukocyte alkaline phosphatase and catalase concentration in a 24 year old woman with chronic granulocytic leukemia. N-APA and CAA represent the biochemically determined alkaline phosphatase and catalase activities of leukocytes respectively.

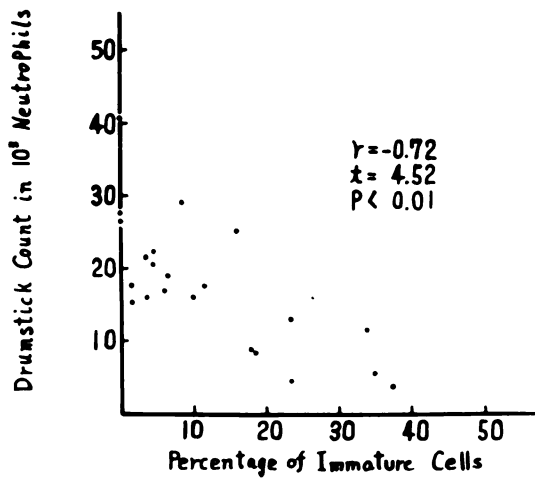


Fig. 6.—The relationship between the drumstick counts and the percentage of immature cells in peripheral blood in various stages of eight female cases of chronic granulocytic leukemia.

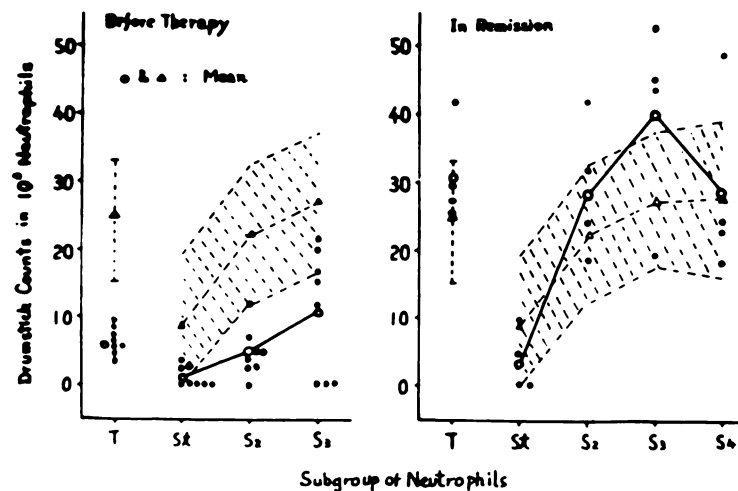


Fig. 7.—The relationship between the drumstick counts and the lobe counts of neutrophils in women with chronic granulocytic leukemia. The dotted lines represent the mean  $\pm$  one standard deviation of 100 normal female subjects. St = band-forms of neutrophils,  $S_n$  = segmented neutrophils with  $n$  lobes, and  $T = St + S$ .

to therapy the number of sessile nodules and small clubs was moderately reduced in the neutrophils from many of the patients with these disorders (table 3). In both CGL and inflammatory leukocytosis the counts returned to normal following treatment (fig. 3).

#### DISCUSSION

Davidson's observation of the drumstick as sex chromatin has been confirmed many times in its essential points,<sup>3,10,12,14,17</sup> although some have questioned the relationship between sex chromatin and sex chromosome.<sup>18,23</sup> Since the drumstick counts show wide variation in normal women and some cells bear drumsticks while others do not, one must qualify conclusions from observations made from the drumstick counts in patients. It is reasonable to assume, however, that the drumstick count is the product of many interrelated factors, as the distribution of drumstick counts is represented by a bell-shaped curve (fig. 2). The drumstick count for each individual is so constant that its enumeration provides a method for differentiating women from one another within certain limits.<sup>3</sup> This suggests that the occurrence of drumsticks might be the consequence of some underlying biological arrangement which persists throughout life and may not depend upon changes in the physiologic climate. The fact that the drumstick counts of the patients with infections in this study remained within the normal range may support this concept. On the other hand, the remarkable changes of the drumstick counts in CGL give rise to certain speculations with regard to the leukemic process, since the leukemias probably arise from disturbances within cell nuclei.

When the various appendages of nuclei of polymorphonuclear neutrophils were investigated in CGL, leukemoid reactions, and inflammatory leukocyto-

ses, only the changes in the drumstick counts in CGL showed any striking differences. Although the number of cases of CGL studied was small, this alteration was constant, and may be characteristic for CGL. The drumstick counts of the neutrophils in CGL are abnormally low when the disease is in relapse, increase rapidly during treatment, and return to normal in remission. These counts parallel the changes observed in leukocyte alkaline phosphatase and catalase with treatment. This phenomenon could be explained if it is assumed that leukemic neutrophils have a reduced capacity to develop drumsticks, and it is these same cells that show the enzyme change. Only a portion of the total circulating granulocyte population is affected in this way. These observations lead to the suggestion that there may be two cell populations in chronic granulocytic leukemia, one with abnormal and another with normal chromosomal and enzymatic characteristics.

In recent years, chromosome analyses have been performed in the cells from a wide range of mammalian tumors. Changes in chromosome structure, in chromosome number, and in amount of DNA have been reported in transplanted tumors<sup>7,13,15,16,23</sup> and in neoplasms induced by chemicals<sup>8,20</sup> and radiation.<sup>5</sup> Wakonig<sup>22</sup> performed chromosome analyses on spontaneous leukemia of AKR mice and reported that most of the 17 mice with primary leukemia had modal chromosome numbers of 40 (diploid). In five animals the mode was 41 and one had 2 modes. Similar cytologic changes also were noted in human leukemias. Ford<sup>6</sup> found chromosomal abnormalities in four of six cases of blast cell leukemia, but no abnormalities in each of two cases of chronic lymphocytic leukemia and CGL. Baikie<sup>2</sup> reported that acute leukemia consisted of cells having aneuploidy or diploidy with chromosomal alterations, but that chronic leukemias had a normal diploid chromosome complement. Awano<sup>1</sup> demonstrated by means of detailed comparison of chromosomal aberrations in acute and chronic granulocytic leukemia with normal cells that the variation in number of chromosomes was larger in leukemic cells than in normal somatic cells. He also showed that the large L-shaped chromosomes, corresponding to the X element, were not present in most leukemic cells, but frequently there was an increase in small rod-shaped chromosomes.

If we want to discuss the abnormal reduction of the drumstick counts in CGL which were observed in this study, directly in relation to the studies on chromosomes in human leukemias and to regard such reduction as a reflection of the alteration of chromosomes, especially sex chromosomes, it would be necessary to perform parallel analyses of drumstick and chromosome alterations in details in many more cases of CGL and other pathologic conditions and to determine whether the reduction of the drumstick counts is really pathognomonic in CGL.

From this study it is not possible to be certain that the reduction in the number of drumsticks found in the neutrophils of patients with CGL is a direct reflection of the chromosomal alterations, especially sex chromosomes, known to exist in certain leukemic cells. In order to establish this relationship it would be necessary to perform concomitant drumstick and chromosome analyses in many more humans with a variety of clinical disorders. Of particu-



lar importance would be those clinical states characterized by sexual dysgenesis. The results of the present study, however, indicate that analyses of this type may have interesting potentialities. At least, it may be said that the results of this morphologic study support the double cell population theory developed by means of leukocyte enzyme studies.

#### SUMMARY

1. The drumstick and other nuclear appendages of the nuclei of polymorphonuclear neutrophils from patients with chronic granulocytic leukemia (CGL), leukemoid reactions and inflammatory leukocytoses were studied.

2. The drumstick counts of the leukocytes of patients with CGL were abnormally low. In contrast, the counts in the leukocytes from patients with the other disorders were not reduced. The low counts increased rapidly in response to treatment and were maintained at normal levels during remission.

3. The normalization of drumstick counts in CGL paralleled the leukocyte alkaline phosphatase and catalase concentrations.

4. The number of sessile nodules and small clubs in each of the disorders studied was moderately reduced. Following treatment, the counts returned to normal. This suggests a relationship between the development of these appendages and hyperproliferation of neutrophils.

#### SUMMARIO IN INTERLINGUA

1. Esseva studiate le "baston de tambur" e altere appendiculas nucleari in le neutrophilos polymorphonucleari ab patientes con chronic leucemia granulocytic, con reacciones leucemoide, o con leucocytoses inflammatori.

2. Le numeration del "bastones de tambur" in patientes con chronic leucemia granulocytic esseva anormalmente basse. Per contrasto con isto, le correspondent numerationes in le caso del patientes con le altere supramentionate disordines non esseva reducite. Le basse numerationes in le patientes con chronic leucemia granulocytic montava rapidamente in responsa al tractamento e se manteneva a nivellos normal durante intervallos de remission.

3. Le normalisation del numeration de "bastones de tambur" in patientes con chronic leucemia granulocytic progrededa in parallela al normalisation del concentration de phosphatase alcalin e de catalase in le leucocytos.

4. Le numeration del nodulos sessile e del micre fustes in le varie disordines studiate esseva moderatemente reducite. Post tractamento, iste numerationes retornava al norma. Le observationes suggere le existentia de un relation inter le disveloppamento del appendiculas nucleari e le hyperproliferation de neutrophilos.

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