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Comparison of morphological changes in white blood cells after death and in vitro storage of blood for the estimation of postmortem interval

H. Dokgöz[a], N. Arican[b], I. Elmas[b,*], S.K. Fincanci[b]

[a]*The Council of Forensic Medicine, Istanbul, Turkey*

[b]*Department of Forensic Medicine, Istanbul University, Istanbul Medical Faculty, Istanbul, Turkey*

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Abstract

Estimation of the time of death is one of the most important problems for forensic medicine and law. Physical and chemical postmortem changes are evaluated together while estimating the time of death.

In this study, in vitro storage and postmortem changes of white blood cells were aimed to be compared within the given postmortem interval, and a follow-up study was carried out. Blood smears which were obtained from 10 non-refrigerated cadavers (experimental group) and from 40 hospital patients (control group) have been evaluated to observe and compare changes during the in vitro storage and postmortem degenerative morphological changes that white blood cells undergo throughout the given postmortem intervals. The samples were examined by using a light microscope, and blood cells were differentiated by staining blood films with May–Grunwald stain, followed by Giemsa stain. Identifiable degenerated eosinophils and monocytes were first examined at 6 h of death and the in vitro storage, and they were unidentifiable beyond 72 h of storage. Identifiable degeneration of neutrophils were first examined at 6 h of death and storage while unidentifiable beyond 96 h of storage. Identifiable degeneration of lymphocytes were first examined at 24 h of death, and they were still identifiable beyond 120 h.

Cellular changes of leukocytes can be useful in the 6–120 h for estimating the time of in vitro storage, and the findings match during the first 21 h for both experimental and control groups. Finally, this follow-up study and the comparison will also be carried out for a longer postmortem interval, and other specific hypothesis that relate cellular changes in tissues other than blood with time since death are various points that needs to be studied.

Keywords: Postmortem interval; Cellular changes; Leukocyte

1. Introduction

The basic characteristics which are necessary for the methods to estimate the time of death are their being easy to apply and non-invasive. It is easy to obtain postmortem blood samples even at the site of the scene and it takes not more than a few minutes. The main problem is to provide a reliable method that could be based on blood analysis. There are many researches for estimation of postmortem interval. Querido showed the in vitro potassium loss from erythrocytes and the increase of serum potassium concentration as a result of it, the linear correlation between sodium and potassium concentrations, and the changes in plasma chloride concentration, sodium and calcium concentration, and determination of hormones in sera were also carried out [1–4]. Blood glucose, lactic acid, non-protein nitrogen, ammonia, cholesterol, lipid and protein, electrolytes, hormones, alkaline phosphatase, transaminase, and amylase, and other chemical substances were searched [5,6]. Researches are held for the separation rate of the third component of the complement [7,8]. None of these methods were found useful alone in forensic sciences.

* Corresponding author. Present address: Department of Forensic Medicine, Istanbul University, Istanbul Medical Faculty, 34390 Capa-Istanbul, Turkey. Tel.: +90-212-635-1179; fax: +90-212-635-8522.
E-mail address: imdate@istanbul.edu.tr (I. Elmas).

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In this study, the morphological changes observed in leukocytes as a result of autolysis and putrefaction, and relation of these changes with the given postmortem interval itself is compared. Estimation of postmortem interval according to these degenerative changes is discussed by reviewing the literature. A brief report of this study has appeared in abstract form [9].

2. Material and methods

This study was performed on blood samples that have been collected from 10 non-refrigerated cadavers (experimental group) whose time of death was known and who were between 20 and 40 ages without any oncological, haematological or infectious diseases, and from 40 hospital patients (control group) of the same ages.

Blood samples of 2 ml each from the non-refrigerated cadavers, of which the blood samples could be maintained within 21 h of postmortem interval, were drawn from internal jugular vein, and as for the hospital patients from their vena cava cubiti, and put in sterile glass bottles with rubber bungs, in which 0.2 ml of a 10% solution ethylene diamine tetraacetic acid (EDTA) K_3 as anticoagulant had been added. The samples that were drawn from hospital patients were labeled, numerated and were kept at 24–26°C room temperature which had been similar to the room temperature of which the cadavers (experimental group) had been kept for 0–21 h time period. Thus, the cadavers could not be kept more than 21 h, and the blood samples from the cadavers were stored in vitro while a blood smear was prepared prior to in vitro storage with a label of the given postmortem interval. The blood smears of cadaveric group had been prepared immediately after being drawn, and labeled according to the time of death of the cadavers. The findings are compared with the findings of the same in vitro period of control group. Subsamples are taken from the blood samples of both groups after 0, 3, 6, 9, 12, 18, 24, 36, 48, 60, 72, 84, 96, and 120 h of storage, and peripheral blood films of both the experimental and control groups were prepared by staining with May–Grunwald and Giemsa stain. They were washed by newly made distilled water after 15 min, and left to dry. The peripheral smears were examined under the light-microscope in an area of 1 cm × 2 cm which was presumed to contain approximately 100 cells. The nuclear and cytoplasmic morphological changes in leukocytes have been recorded. The stained smears and findings were cross-checked by consultant haematologists. The cell changes

Table 1
Morphological changes of leukocytes in a unit area of blood smear of the experimental and control subjects

Cell types	Cell changes	Time (h)
Neutrophils	Pyknosis	>6
	Cytoplasmic and nuclear vacuolation	>12
	Nuclear fragmentation	>18
	Disintegration	48–96
Eosinophils	Pyknosis	>6
	Cytoplasmic and nuclear vacuolation	>12
	Nuclear fragmentation	>18
	Disintegration	48–72
Monocytes	Pyknosis	>6
	Cytoplasmic and nuclear vacuolation	>12
	Nuclear fragmentation	>24
	Disintegration	48–72
Lymphocytes	Nucleus swollen, cytoplasm and cell membrane indistinct	>24
	Pyknosis	>36
	Nuclear fragmentation	>72
	Disintegration	96 ↑

Table 2
Time of death and cell changes[a,b]

Case no.	PMI (h)	Neutrophils				Eosinophils				Monocytes				Lymphocytes				
		P	C	N	D	P	C	N	D	P	C	N	D	S	P	C	N	D
1	12	+	+	−	−	+	+	−	−	+	−	−	−	−	−	−	−	
2	18	+	+	+	−	+	+	+	−	+	+	−	−	−	−	−	−	−
3	9	+	−	−	−	+	−	−	−	+	−	−	−	−	−	−	−	−
4	3	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
5	21	+	+	+	−	+	+	+	−	+	+	−	−	−	−	−	−	−
6	3	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
7	6	+	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−
8	0	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
9	3	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
10	0	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−

[a] P: pyknosis; S: nucleus swollen, cytoplasm and cell membrane indistinct; C: cytoplasmic and nuclear vacuolation; N: nuclear fragmentation; D: disintegration.

[b] Blood smears of cadaveric group revealed similar cellular changes compared with the control group according to the time of death of which the cadaveric samples had been collected.

of the subsamples of cadaveric blood had also been followed and they had been labeled by adding the period of in vitro storage to the given postmortem interval. The results were shown in tables and figures and a blood cell variation scale (BCVS) has been prepared.

3. Results

Similar morphological changes shown in Tables 1 and 2 as a result of the examination of the peripheral smears prepared from the blood samples taken from non-refrigerated cadavers and hospital patients group periodically in 21 h postmortem interval, and 120 h total storage time are observed. Identifiable degeneration of neutrophils, eosinophils and monocytes were first examined at 6 h for both groups, and the findings for postmortem and in vitro storage interval were similar. The degenerative changes of lymphocytes began after 24 h of in vitro storage, and the findings of both groups matched for the first 21 h of which the blood samples could be maintained from the non-refrigerated cadavers with a given postmortem interval. Cellular changes

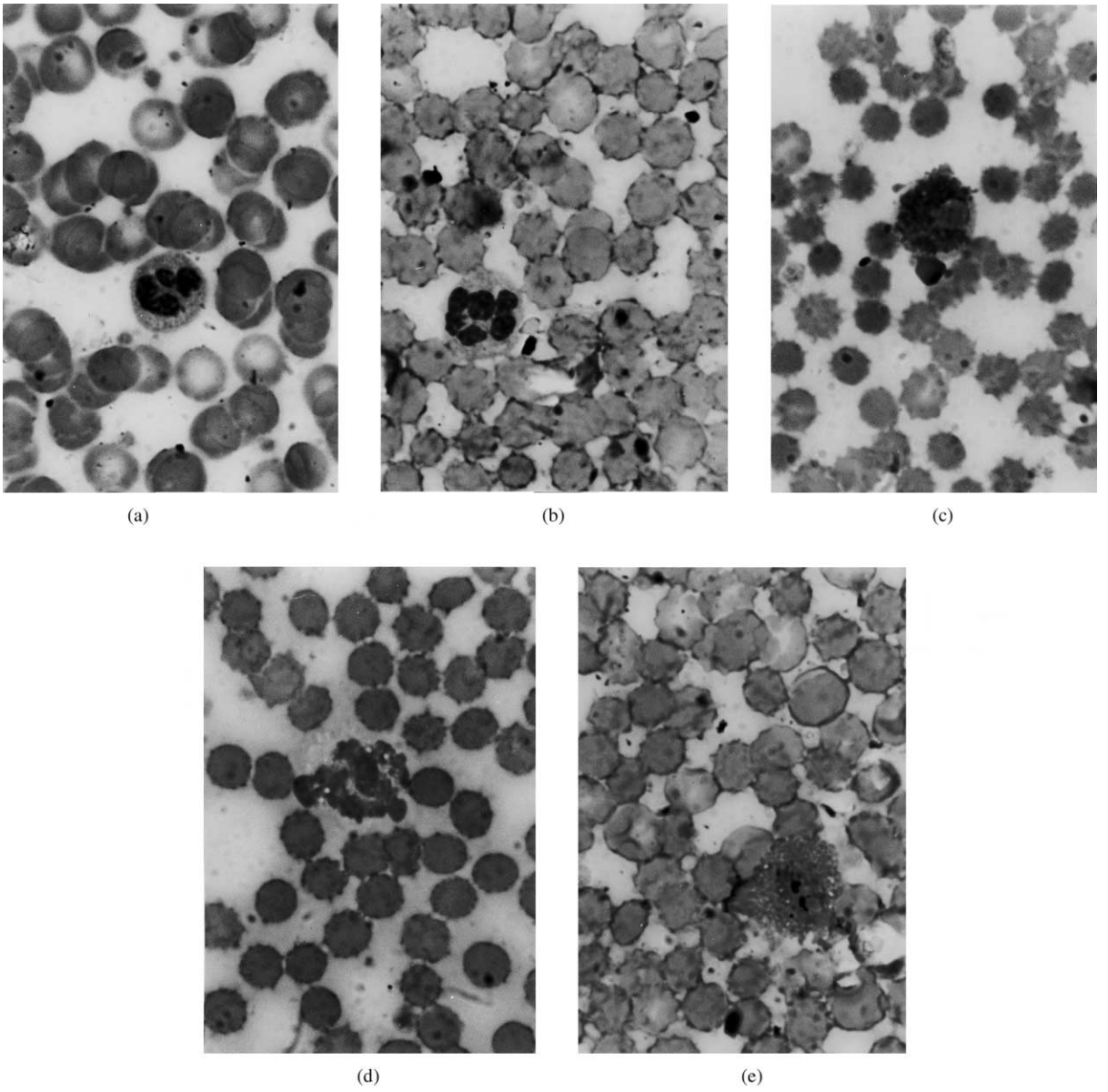

Fig. 1. Degenerative morphological changes of neutrophils. (a) Normal; (b) pyknosis; (c) cytoplasmic and nuclear vacuolation; (d) nuclear fragmentation; (e) disintegration HE, 1000×.

had also lead to the same result while the subsamples of cadaveric group had been observed in vitro. Degenerated neutrophils were unidentifiable beyond 96 h in vitro, and eosinophils and monocytes were unidentifiable beyond 72 h after in vitro storage. Lymphocytes were still identifiable beyond 120 h and later. In vitro storage of the blood samples which had been driven from the cadavers were also compared with the control samples, and the findings were similar for the 120 h in vitro follow-up of both groups. The basophils are not taken into account in this study for their rarely observance in peripheral smears researches. The morphological changes (b–e) which have been seen in neutrophils, eosinophils, monocytes and lymphocytes are shown in Figs. 1–4. The morphological changes which have been observed during in vitro storage were marked at a BCVS in relation with the time period, and these findings are shown in Fig. 5. BCVS points out the in vitro morphological changes, however, the postmortem changes of cadaveric blood samples matched with the in vitro changes, and follow-up in vitro study of cadaveric samples which had been labeled by calculating PMI + in vitro storage have also revealed similar results with the control group.

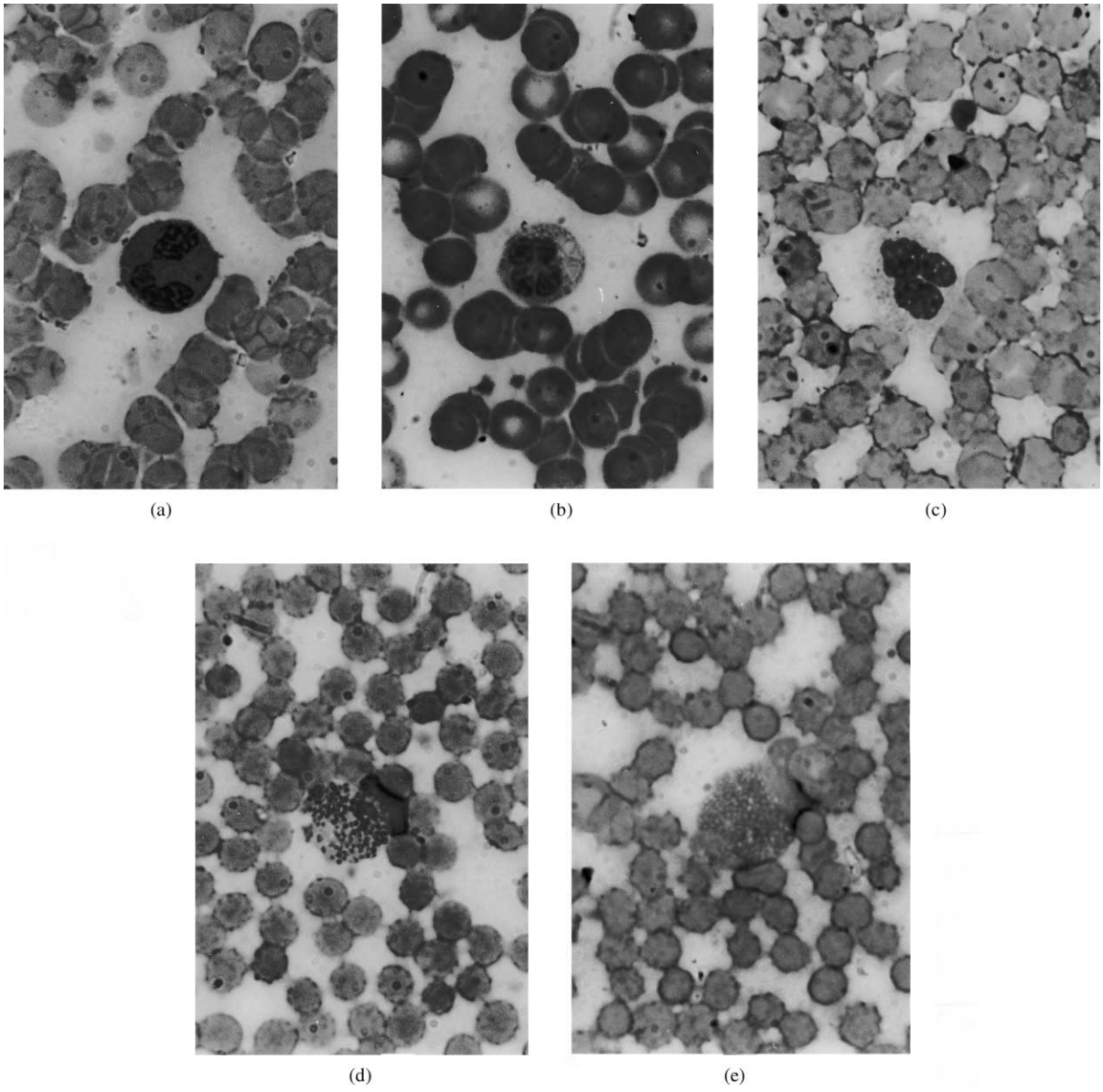

Fig. 2. Degenerative morphological changes of eosinophils.(a) Normal; (b) pyknosis; (c) cytoplasmic and nuclear vacuolation; (d) nuclear fragmentation; (e) disintegration HE, 1000×.

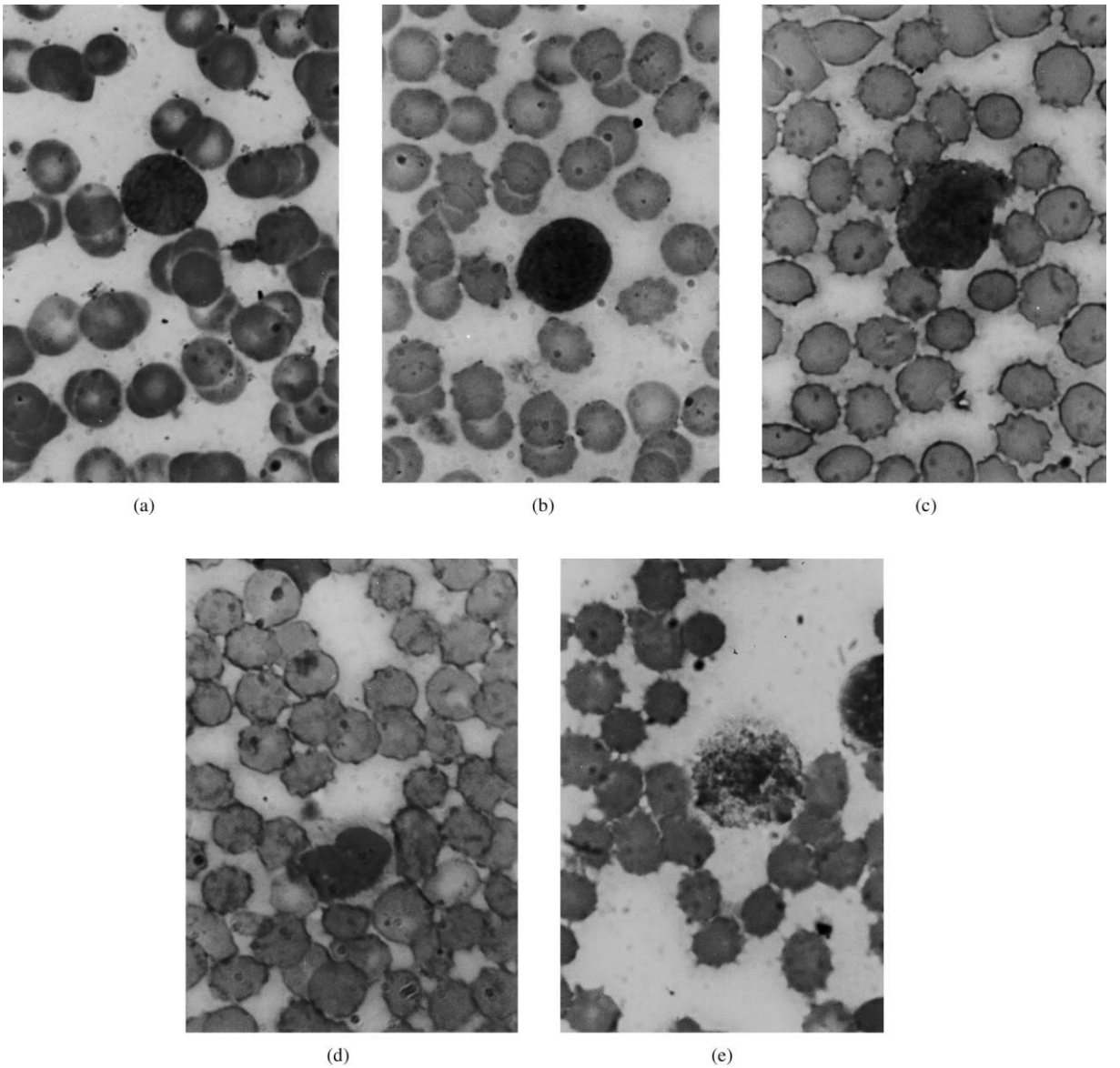

Fig. 3. Degenerative morphological changes of monocytes. (a) Normal; (b) pyknosis; (c) cytoplasmic and nuclear vacuolation; (d) nuclear fragmentation; (e) disintegration HE, 1000×.

4. Discussion and conclusion

Like other tissue cells, blood cells also lose their normal morphology as a result of postmortem autolysis and putrefaction, and are unidentifiable in the last period. The changing process through normal morphology to unidentification period can be a useful criterion for estimating postmortem interval [10].

In this study, the findings indicated that neutrophils, eosinophils and monocytes did not lose their normal morphology during the first 6 h, and neither did the lymphocytes during the first 24 h. Babapulle and Jayasundera reported that the cells of the blood samples which they examined for 144 h were normal in the first 6 h, were normal and abnormal between the first 6 and 72 h, and were all abnormal and difficult to identify in morphology [11].

In this research, we observed pycnosis after the first 6 h and cytoplasmic and nuclear vacuolation after 12 h in neutrophils, eosinophils and monocytes. Nuclear fragmentation started after 18 h in neutrophils and eosinophils and after 24 h in monocytes. Disintegration was observed between 48 and 96 h in neutrophils, and between 48 and 72 h in eosinophils and monocytes. In lymphocytes nuclear swelling and indistinctness in cytoplasmic cell membrane were

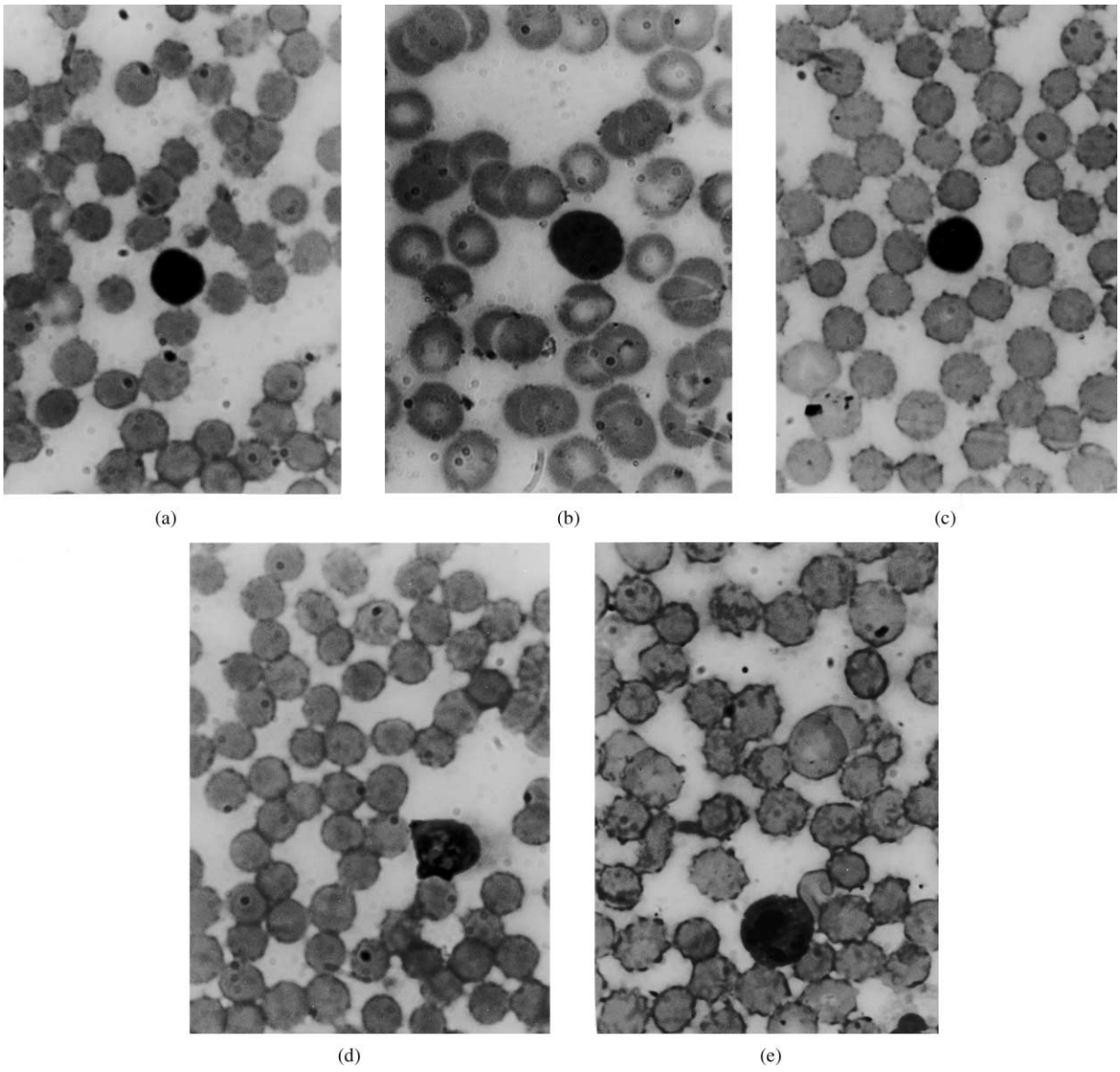

Fig. 4. Degenerative morphological changes of lymphocytes. (a) Normal; (b) nucleus swollen, cytoplasm and cell membrane indistinct; (c) pyknosis; (d) nuclear fragmentation; (e) disintegration HE, 1000×.

observed after 24 h, while pycnosis, nuclear fragmentation and disintegration were observed after 36, 72 and 96 h, respectively. Lymphocytes were still identifiable after 120 h and later (Table 1, Figs. 1–4).

Experimental group results matched the results of in vitro storage blood samples during the first 21 h, and in vitro storage results of both groups were also similar during the follow-up. The facts that the cells kept their normal morphology during certain periods and that they had the degenerative changes described above and that they were unidentifiable later, provided the basis of the blood cell variation scale which is based on those parameters. Only a broad estimation of in vitro storage can be made by this scale although blood cell changes matched for both group and in a future research for comparison it is planned to study on a larger group of non-refrigerated cadavers with a longer given postmortem interval to apply the scale for determination of postmortem interval (Fig. 5). The studies on the morphological and chemical assessment of the postmortem interval have revealed that the speed of all postmortem changes varies widely in dependence of the conditions of storage during the postmortem interval [12–15]. It should be noted that, it is quite difficult to report the exact time of death because of many factors [12–16]. Although there are many

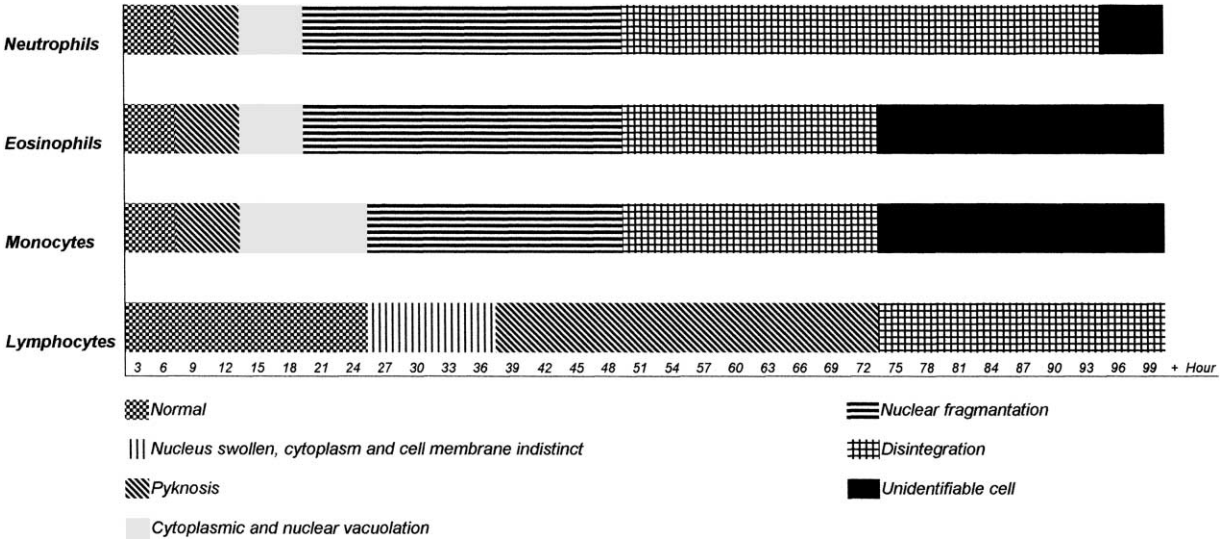

Fig. 5. Blood cell variation scale (BCVS). The scale is drawn according to the data collected from in vitro cell changes of control group blood samples.

methods to estimate the time of death in forensic medicine, none of them is reliable enough by itself. Therefore, many of the available methods are applied together and the possible right time is estimated. The estimation of the time of death according to the morphological changes in leukocytes should be accepted in this context.

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