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LIVER STEATOSIS IN BRAIN DEATH DONORS

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Objective: to study the frequency of fatty hepatosis in liver biopsies of consecutive brain death donors before cold preservation. **Materials and methods.** Liver biopsies (before cold preservation) of 300 consecutive donors with brain death were studied. Histological preparations were stained with hematoxylin and eosin, and tricolor Masson staining was performed. **Results.** The frequency of different degrees of fat hepatosis in men and women did not differ significantly (>0.05). Fat dystrophy of hepatocytes was absent in more than half of the cases (n = 182; 60.7%). A slight degree of fatty degeneration was diagnosed in 57 (19,0%) donors. In total, 239 (79.7%) donor livers were absolutely suitable for transplantation. Moderate degree of steatosis, which is associated with early biliary complications, was detected in 18 (6.0%) cases, and severe degree, which is a contraindication to the use of the organ for transplantation, was detected in 43 (14.3%) cases. **Conclusion.** Before cold preservation, liver from brain death donors is relatively rarely unsuitable for transplantation.

Keywords: donor liver, biopsy, steatosis.

INTRODUCTION

Donor liver steatosis is one of the important morphological criteria to determine the suitability of an organ for transplantation. With an increase in the number of patients with diabetes and obesity, non-alcoholic fatty liver disease is becoming more common, affecting a quarter of adults worldwide [1]. This disease is manifested either as simple steatosis or non-alcoholic steatohepatitis [2]. Currently, donor liver with steatosis has been used for transplantation. The use of steatous liver is associated with both a lack of donor organs and an increase in the prevalence of fatty liver disease in the general population [3, 4].

Increase in the degree of macrovesicular steatosis of the donor liver above 30% is well known to associate with the risk of increased reperfusion damage, thus increasing the incidence of primary non-functioning graft and its lower survival [1, 5, 6]. However, there is more and more evidence that, with careful selection of recipients, donor liver with moderate and severe macrovesicular steatosis can be successfully used for transplantation [1, 5, 6]. After liver transplantation with steatosis, a decrease in its degree is observed [5].

However, donor liver with steatosis is more susceptible to ischemic damage during cold preservation [7]. Such organs are poorly restored after transplantation. Ischemic and reperfusion injury disrupts microcirculation due to disruption of the vascular endothelial lining [8], increases oxidative damage to mitochondria, and also increases neutrophil aggregation and leads to an imbalance in cytokine release [6]. All this increases the risk of organ dysfunction after transplantation [7].

Therapeutic approaches to expand the use of steatosis donor liver transplantation are currently being intensively studied. Preconditioning of the donor liver can reduce the accumulation of xanthine and suppress the activity of xanthine oxidase, which increases with steatosis during cold ischemia, and thereby protect it from damage. Several pharmacological agents have also been shown to be effective in protecting donor liver with steatosis from ischemic and reperfusion damage.

The present study was aimed at investigating the nature and degree of steatosis in the liver of brain death donors before its cold preservation.

MATERIALS AND METHODS

The liver biopsies of 300 consecutive brain death donors were histologically examined. The biopsies were performed prior to cold preservation of the donor liver. Biopsies were fixed in 10% neutral formalin and embedded in paraffin. From paraffin blocks, histological sections with a thickness of 4–5 μm were prepared, which were stained with hematoxylin and eosin, as well as by Masson's method. The preparations were studied in the bright field of a Leica DM 6000B microscope. Statistical processing of the results was carried out using the statis-

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tical software package Statistica 7.0 (StatSoft, USA) and MS Office EXCEL (Microsoft, USA). The significance of the differences was assessed by the Student's test. Differences were considered significant at $p \leq 0.05$.

RESULTS AND DISCUSSION

Hepatic steatosis was qualitatively assessed on the basis of determining the size of fatty vacuoles in the cytoplasm of hepatocytes. Microvesicular, medium-vesicular, and macrovesicular steatosis were distinguished. Many researchers [9] do not differentiate medium droplet steatosis. However, in our opinion, it is advisable to do this, since in case of medium-drop fatty hepatosis, fatty degeneration of hepatocytes occurs, but they remain viable and after the cessation of exposure to damaging factors (often after liver transplantation), their complete repair occurs.

The presence of only small vesicles in the liver in the cytoplasm of hepatocytes, even if their diffuse distribution took place, in our opinion, is not fatty hepatosis, but is a simple fatty infiltration, which has a transient nature and, most often, alimentary origin. According to the literature [10], the function of hepatocytes is preserved.

In macrovesicular steatosis, large fatty vesicles occupy almost the entire space of the cytoplasm, pushing the nucleus to the periphery of hepatocytes. They are in a state of parabiosis, and, more often than not, their death occurs.

The severity of hepatic steatosis is semi-quantitatively estimated by the percentage of hepatocytes containing lipids [11]. Usually, with steatosis, vesicles of various sizes (small, medium and large) are present in the cytoplasm of hepatocytes in the liver. Therefore, we determined the severity of fatty hepatosis by the number of hepatocytes in which medium- or macrovesicular fatty degeneration prevailed.

The presence of fatty vesicles in less than 5% of hepatocytes, we, like other researchers [11], did not refer to the category of steatous liver. Mild fatty hepatosis was diagnosed on the condition that from 5% to 30% of hepatocytes contained medium-vesicular and/or macrovesicular fatty vacuoles. Such a liver is quite suitable for transplantation, provided that there are no other pathological processes in it. The case is presented of our own observation. Donor D., woman of 62 (biopsy No. 7010-18 of 09.08.19). Death occurred due to acute cerebrovascular accident of the hemorrhagic type. Some biochemical parameters at the time of biopsy were as follows: bilirubin 11, 7; ALT/AST = 21/23. The liver is of average size, yellow color, with rounded edges. Histology revealed coarse fatty degeneration in less than 30% of hepatocytes (Fig. 1). Conclusion: mild fatty hepatosis, no fibrosis (F0).

Unfortunately, a mild degree of fatty hepatosis can be combined with another pathology, which casts doubt on the possibility of its transplantation. For example, donor S., a man of 46 (biopsy No. 7277-79 of 16.08.19), whose death occurred due to acute cerebrovascular accident of the hemorrhagic type (bilirubin 38.9; ALT/AST = 16.8/13.3); the liver of average size, gray-brown color, with sharp edges. Histology revealed macrovesicular fatty degeneration in less than 30% of hepatocytes mainly located in the periportal zones. However, in addition, many hepatocytes had sandy nuclei, which is a manifestation of severe dystrophy. The portal tracts were fibrosed with multiple septa (Figs. 2, 3). Based on this, the following conclusion was made: mild fatty hepatosis, hepatocyte dystrophy, moderate liver fibrosis (F2).

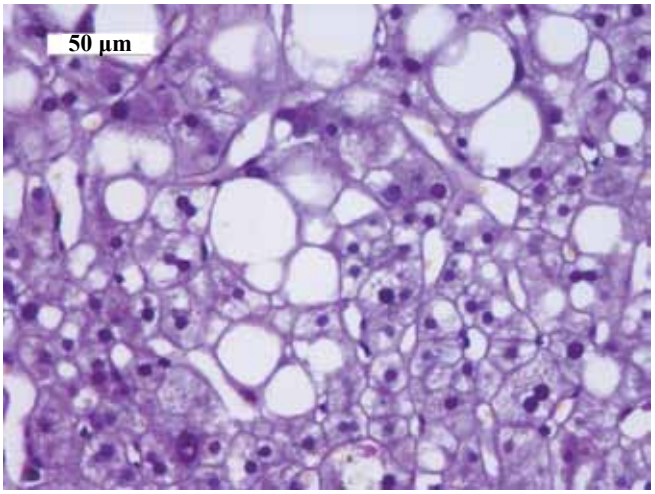

Fig. 1. Donor D., female, 62. In the picture: a small group of macrovesicular fatty dystrophy of hepatocytes. Masson's trichrome stain. Microscope, ×40

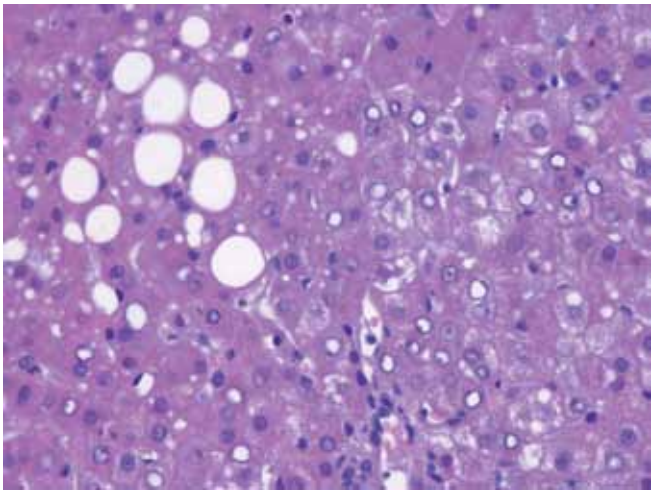

Fig. 2. Donor S., male, 46. In the picture: a small group of macrovesicular fatty dystrophy of hepatocytes. A large number of hepatocytes with sand poisons. Masson's trichrome stain. Microscope, ×40

Moderate (medium) degree of fatty hepatosis was diagnosed in the presence of 30% to 60% of hepatocytes with fatty vacuoles. Moderate, especially moderate vesicular, steatosis is a relative contraindication for liver transplantation. Donor P., male of 56 (biopsy No. 5644-45 of 03.10.17). Death of the brain as a result of closed traumatic brain injury. Total bilirubin 41.1, AST/ALT = 18.2/47.0. The liver of normal size, yellow-gray color, with moderate swelling, rounded edges. Histology revealed large-drop fatty degeneration in less than 60% of hepatocytes (Figs. 4, 5). Mild liver fibrosis. Conclusion. Moderate degree of coarse fatty hepatosis. F1.

Moderate steatosis is a relative contraindication for liver transplantation unless other lesions are present in the liver biopsy. Here is one of our observations, in which there was a combination of a moderate degree of medium vesicular steatosis with a severe degree of ischemic damage. Donor R., male of 33 (biopsy No. 1217-19 of 02/08/19). Brain death occurred as a result of a closed traumatic brain injury. Total bilirubin –20.2. AST/ALT = 2200/1990. The results of histological examination showed a violation of the beam and lobular structure of the liver. Large foci (up to 50% of the area of the preparation) of severe balloon dystrophy and necrosis of hepatocytes. Fatty degeneration of other hepatocytes (Figs. 6, 7). Conclusion: severe ischemic liver damage; moderate degree of medium vesicular fatty hepatosis; no fibrosis (F0).

If the number of hepatocytes with obesity was 60% or more, then such a liver was classified as severe fatty

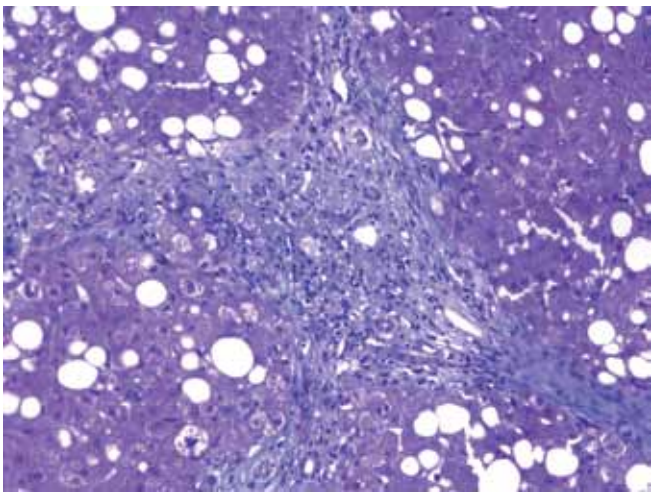

Fig. 3. The same specimen. In the picture: sclerosed portal tract with septa. In periportal hepatocytes – polymorphic fatty dystrophy. Masson's trichrome stain. Microscope, ×40

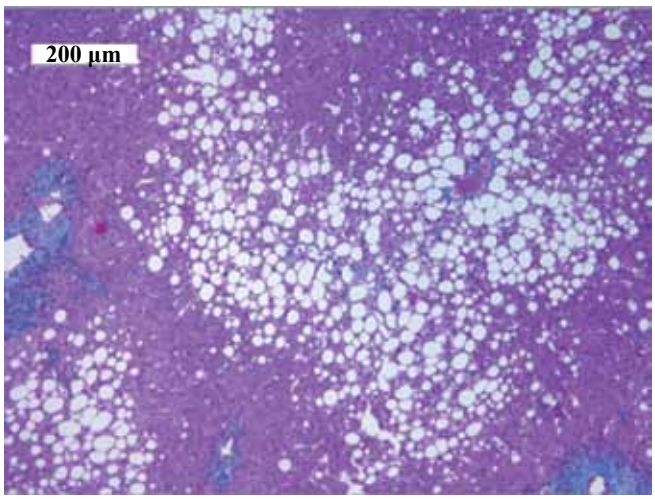

Fig. 4. Polymorphic fatty dystrophy in less than 60% of hepatocytes. Masson's trichrome stain. Microscope, ×10

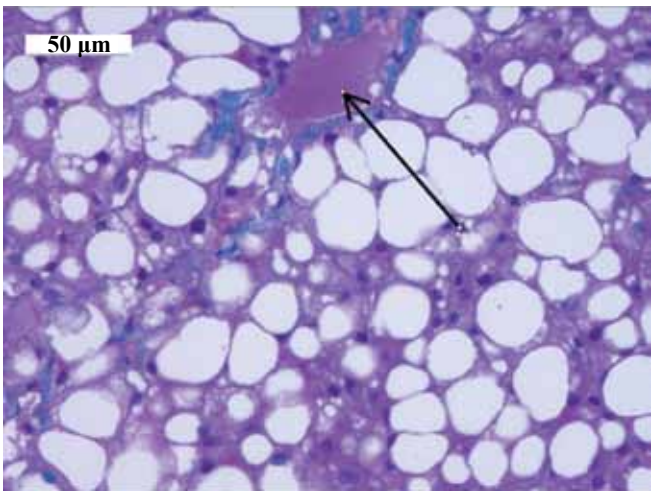

Fig. 5. The same specimen. Polymorphic fatty dystrophy of hepatocytes under high magnification of the microscope. The arrow indicates the central vein. Microscope, ×40

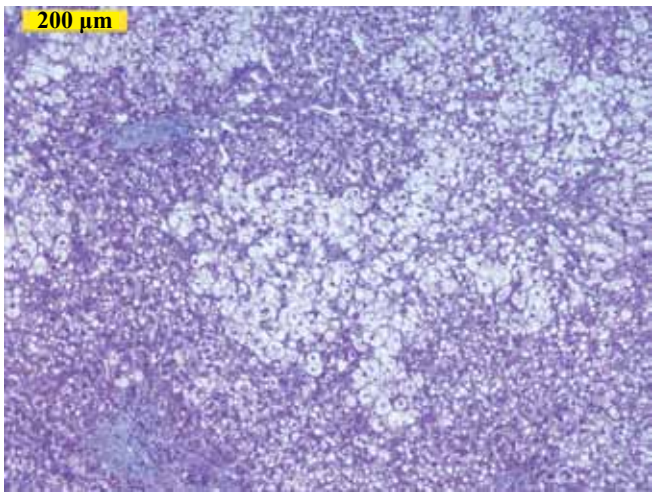

Fig. 6. The focus of balloon dystrophy of hepatocytes, on the periphery of which medium-drop fatty dystrophy is less than 60% of hepatocytes. Masson's trichrome stain. Microscope, ×10

hepatosis. Donor L., male of 50 (biopsy No. 7019-21 of 08/09/19). Brain death occurred due to acute hemorrhagic disorders of cerebral circulation. Basic clinical data (at the time of biopsy): ALT/AST = 47/89, bilirubin 20.3. The enlarged liver, of yellow color, with sharp edges. Histology revealed polymorphic fatty degeneration of about 80% of hepatocytes (Fig. 8). Conclusion: severe fatty hepatosis. F0.

With a severe degree of fatty hepatosis, in some cases, fatty hepatosis developed into steatohepatitis. The case of these observations is presented. Donor V., male of 65 (Biopsy No. 7244-52 of 22.08.19). Acute cerebral circulation disorder of hemorrhagic type. Brain death. Bilirubin 46.3, ALT/AST = 50/43. The liver is enlarged, of gray-yellow color. Histology revealed large droplet fatty degeneration in more than 60% of hepatocytes. The rest of the hepatocytes were with hydropic protein dystrophy. The portal tracts are sclerosed with the formation of septa, moderate proliferation of the bile ducts and with pronounced polymorphic leukocyte (mainly mononuclear) infiltration (Fig. 9). Figs. 10 and 11 show the pathology at high magnification. Conclusion: steatohepatitis, moderate liver fibrosis (F2).

The obtained results of the study of various degrees of fatty hepatosis in donors with brain death before cold storage are shown in Fig. 12. Fatty degeneration of hepatocytes was absent in more than half of the observations (n = 182; 60.7%). Mild fatty degeneration, diagnosed in 57 (19.0%) donors, is not a contraindication for liver transplantation. So, initially 239 (79.7%) of 300 donor livers were suitable for transplantation. Moderate degree (II), which is associated with early biliary complications, was identified in 18 (6.0%) cases. Severe degree (III), which is a contraindication to using the organ for trans-

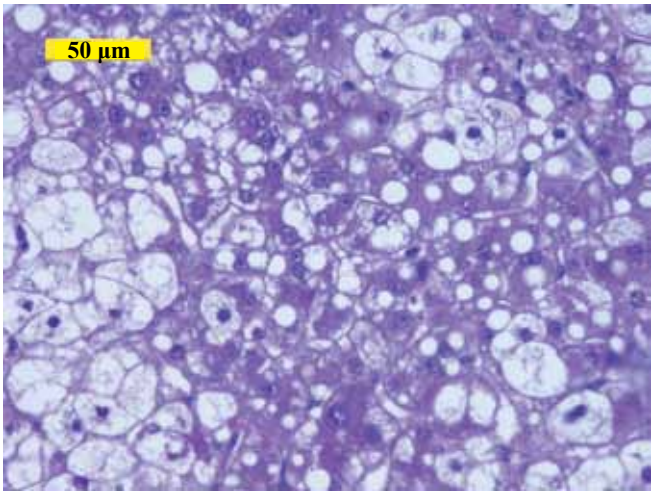

Fig. 7. The same specimen. Microscope, ×40

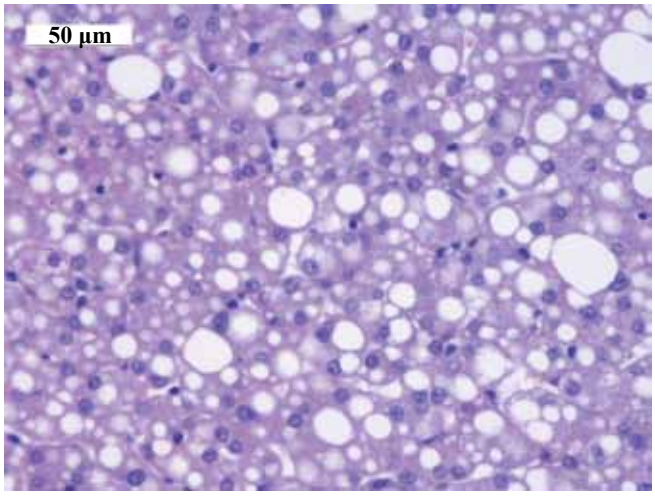

Fig. 8. Polymorphic fatty dystrophy of about 80% of hepatocytes. Masson's trichrome stain. Microscope, ×40

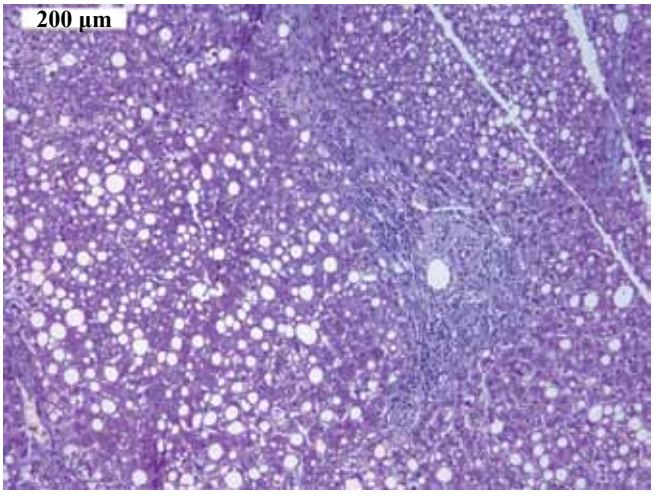

Fig. 9. Polymorphic, mostly middle vesicle fat dystrophy of more than 60% of hepatocytes. Sclerosis of the portal tract with septa. Masson's trichrome stain. Microscope, ×10

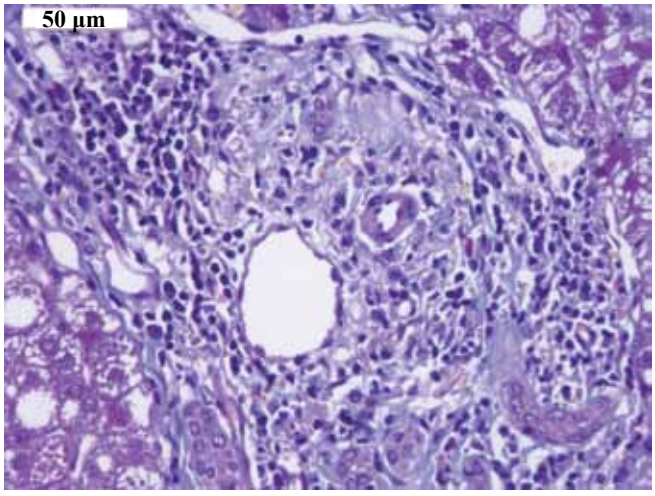

Fig. 10. In the sclerosed portal tract, there is a dense inflammatory infiltration by mononuclear cells. Masson's trichrome stain. Microscope, ×40

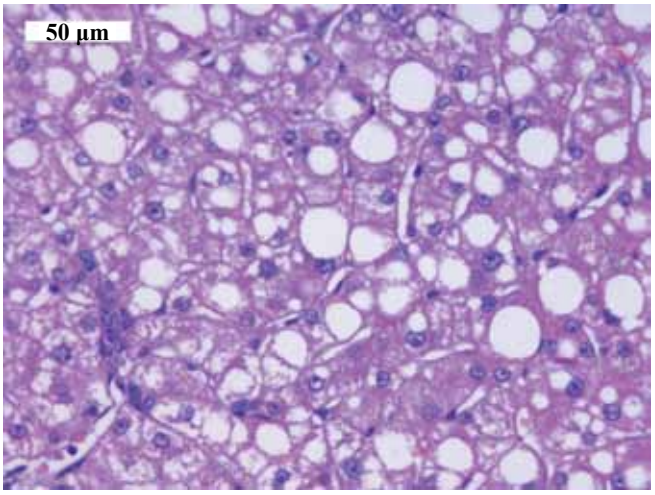

Fig. 11. Polymorphic, mainly medium-vesicular, fat dystrophy of more than 60% of hepatocytes. Masson's trichrome stain. Microscope, ×40

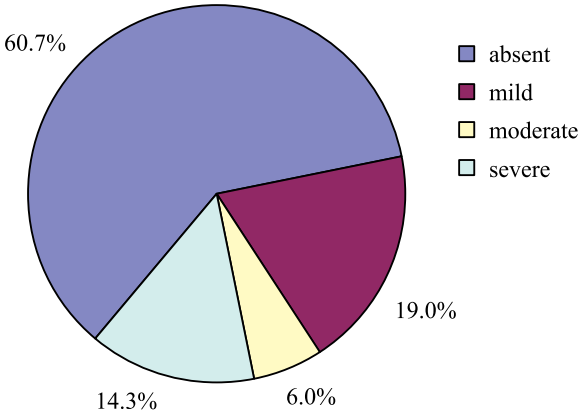

Fig. 12. Various degrees of steatosis in livers of brain death donors before cold preservation

plantation, was diagnosed in 43 (14.3%) donors. The number of donors who belong to donors with extended criteria we attributed the liver with moderate and severe fatty hepatosis (n = 61; 20.3%). The incidence of various degrees of fatty hepatosis in men and women did not differ significantly (>0.05). Therefore, the groups were not divided by gender.

With the increasing demand for donor organs, a higher number of donor livers with steatosis are used in liver transplantation [12]. Recent studies have shown that transplantation of a donor liver with steatosis does not significantly increase the risk of poor outcomes of the transplantation [6]. J.A. Steggerda et al. (2020) [13] raised the threshold limits for the degree of macrovesicular steatosis to 50%. The steatosis degree sharply decreases after liver transplantation [14], which is an additional argument in favor of the possibility of using a donor liver for transplantation with moderate to severe steatosis [6].

CONCLUSION

1. Of the consecutive 300 donors, 239 (79.7%) had no fatty hepatosis or had mild hepatosis, which is not a contraindication for liver transplantation.
2. A relative contraindication to the use of a donor liver for transplantation was in 18 (5%) donors with a moderate degree of steatosis. An absolute contraindication for transplantation is complicated steatosis with inflammation and fibrosis (steatohepatitis).
3. Severe fatty hepatosis was diagnosed in 43 (14.3%) donors. Transplantation of such a liver is permissible in emergencies (urgent surgery) when there is a high risk of near death of the recipient and no more acceptable donor livers.

The authors declare no conflict of interest.

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