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# *Apoptosis of peripheral blood lymphocytes T in nephrotic children*

Apoptosis of activated lymphocytes after completion of their functions is essential for maintenance of human immunity system homeostasis (1, 4, 13). It has been well established that disturbances in the number and function of lymphocytes T play an important role in pathogenesis of the nephrotic syndrome (NS) (6, 8).

The purpose of the study was to assess the intensity of lymphocyte T apoptosis in nephrotic children's peripheral blood before, during and after the treatment with prednisone.

## MATERIAL AND METHODS

The study comprised 26 nephrotic children with first episode, aged 3–14 years (mean 6, 8) hospitalized in the Department of Pediatric Nephrology of University Children Hospital in Lublin. They were treated with prednisone in a dose of 60 mg/m²/24 h. Peripheral blood lymphocytes T were isolated from heparinized blood by gradient centrifugation with Lymphprep (Biocom) before starting steroid therapy (children with acute phase of NS), during steroid therapy – after 4-week therapy (children with a relapse of NS) and after the treatment with prednisone. The controls were 15 healthy children.

The percentages of apoptotic lymphocyte T were determined by flow cytometry using annexin V – fluorescein isothiocyanate (FITC). The mean and standard deviation was determined for each variable in all groups. Comparisons between groups were based on the Mann–Whitney rank test and Kruskal–Wallis test. The significance level was $< 0.05$.

## RESULTS

In acute phase of the NS before starting steroid therapy the mean percentage of apoptotic lymphocytes T was 2.61 (±1.92)%. It was significantly higher than that in controls: 1.08 (± 0.34)%. During treatment with prednisone a statistically significant increase in the mean percentage of apoptotic lymphocytes T was observed. After four-week therapy with prednisone and after completion of the treatment the percentages of apoptotic lymphocytes T were 7.96 (±1.62)% and 9.51 (±4.46)%, respectively. They were significantly higher than those in patients before treatment and in healthy children (Table 1, Fig. 1).

Table 1. Percentage of apoptotic lymphocytes T in nephrotic and healthy children

| Group | Percentage of apoptotic lymphocytes T | | P |
|---|---|---|---|
| | X | SD | |
| 1. Children with acute phase of NS | 2.61 | 1.91 | 1 : 2 $p < 0.01$<br>1 : 3 $p < 0.001$<br>1 : 4 $p < 0.05$<br>2 : 3 $p < 0.05$<br>2 : 4 $p < 0.01$<br>3 : 4 $p < 0.001$ |
| 2. Children after 4 week tratment | 7.96 | 1.62 | |
| 3. Children after completion of the treatment | 9.51 | 4.43 | |
| 4. Healthy children | 1.08 | 0.34 | |

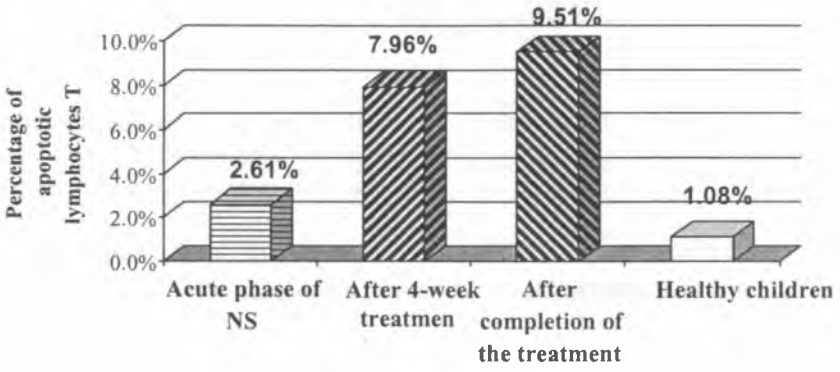


Fig.1. The mean percentages of apoptotic lymphocytes T in nephrotic and healthy children

## DISCUSSION

Many authors have described that the NS was associated by alternation in the number and reactivity of peripheral blood lymphocytes and by disturbances in cytokines and immunoglobulins synthesis, particularly in younger patients (6, 8, 9).

Lymphocytes T activation with subsequent cytokine release into the blood is thought to cause alternation in glomerular filtration barrier permeability and thus it plays an important role in pathogenesis of NS (6). The mechanisms of lymphocytes T activation are still not fully understood. A wide variety of endo-and exogenous factors seems to be involved in this complex process (5, 6).

It has been well established that activated lymphocytes T have been more susceptible to apoptosis than the rest cells. The tight regulation of lymphocytes apoptosis intensity is essential to maintain immune system homeostasis (4, 5, 10, 13).

During programmed cell death the deformation of cell membrane and expression of phosphatidylserine on its surface occur. In contrast to other studies in estimation of the number of apoptotic lymphocytes we used Annexin V which enables the detection of early apoptotic cells most reliably, because it bounds to phospholipids redistributed from cell cytoplasm to cell membrane. The percentage of early apoptotic lymphocytes is a widely accepted marker of lymphocyte apoptosis intensity (1, 3, 10).

In the study acute phase of the NS before prednison therapy institution was characterized by statistically significant increase in the percentage of apoptotic lymphocytes T as compared with controls. This is consistent with the observations made by Z a c h w i e j a et al. (14). However, in contrast to our results, they demonstrated the decrease in the percentage of apoptotic lymphocytes T in children

who obtained remission of the NS and the rate of apoptosis in children with NS in remission was similar to that in the control group. Intensification of lymphocytes T apoptosis due to steroid therapy was also observed by other authors (2, 7, 11, 12). In the study during the treatment with prednisone the gradual increase in the percentage of apoptotic lymphocytes T was noted. After therapy completion the percentage of apoptotic lymphocytes T was significantly higher than those before and during therapy as well as in controls.

Intensification of lymphocytes T apoptosis occurring after prednisone therapy institution may reflect enhanced elimination of their activated forms (11, 12).

A lack of alterations in the intensity of lymphocytes T and B apoptosis during the treatment with prednisone in children with the nephrotic syndrome may suggest the necessity of dosage modification. It seems that the estimation of the intensity of peripheral blood lymphocyte apoptosis will allow to individualize the dosage of prednisone in order to prevent optimally steroid side effects. The monitoring of intensity of lymphocyte apoptosis will also be helpful in the estimation of treatment efficacy.

The assessment of expression of genes regulating apoptosis will be useful in severe or steroid--resistant cases. Overexpression of genes inhibiting apoptosis may suggest significant disturbances of immune system function that require therapy modification.

## CONCLUSIONS

1. In acute phase of the nephrotic syndrome the enhanced intensity of lymphocyte T apoptosis was observed.

2. Prednisone therapy gave rise to further enhancement in the intensity of lymphocyte T apoptosis.

3. Prognostic value of prednisone-induced enhanced intensity of lymphocyte T apoptosis requires further investigations.

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SUMMARY

The purpose of the study was to assess the intensity of lymphocyte T apoptosis in nephrotic children's peripheral blood before, during and after the treatment with prednisone. The percentages of apoptotic lymphocyte T were determined by flow cytometry using annexin V. The study comprised 26 nephrotic children aged 3–14 years. The controls were 15 healthy children. In acute phase of the nephrotic syndrome before institution of the therapy the mean percentage of apoptotic lymphocytes T was 2.61%. It was significantly higher than that in controls (1.08%). During treatment with prednisone a statistically significant increase in the mean percentage of apoptotic lymphocytes T was observed. After four-week therapy with prednisone and after completion of the treatment the percentages of apoptotic lymphocytes T were 7.96% and 9.51%, respectively. They were significantly higher than those in patients before treatment and in healthy children.

Apoptoza limfocytów T we krwi obwodowej u dzieci z zespołem nerczycowym

Celem badań była ocena nasilenia zjawiska apoptozy limfocytów T krwi obwodowej u dzieci z zespołem nerczycowym oraz obserwacja dynamiki zmian w nasileniu apoptozy w trakcie stosowanego leczenia. Badaniami objęto 26 dzieci z zespołem nerczycowym w wieku 3–14 lat. Grupę kontrolną stanowiło 15 zdrowych dzieci. Krew do badań pobierano rano na czczo z żyły łokciowej. Badania przeprowadzano w ostrej fazie choroby przed rozpoczęciem leczenia, w trakcie leczenia prednizonem w dawce 60 mg/m$^2$/24h oraz w okresie remisji po zakończeniu leczenia. Metodą badań była technika cytometrii przepływowej. Komórki krwi izolowano przy pomocy preparatu Lymhoprep. Odsetek i rodzaj limfocytów określano przy użyciu przeciwciał monoklonalnych. Ilość komórek apoptotycznych oznaczano przy użyciu Annexyny V. U dzieci w ostrym okresie choroby, przed rozpoczęciem leczenia, odsetek komórek apoptotycznych limfocytów T wynosił średnio 2,61% i był istotnie wyższy niż w grupie kontrolnej (1,08%). W trakcie leczenia zaobserwowano istotnie statystyczny wzrost odsetka limfocytów T, który po 4 tygodniach sterydoterapii osiągnął wartość 7,96%, a po zakończeniu leczenia – 9,51% i pozostawał na poziomie istotnie wyższym niż w okresie przed rozpoczęciem leczenia i w grupie kontrolnej.