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# Serum and Cerebrospinal Fluid TNF-α in Children with Acute Leukemia of Various Phases

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**Abstract: Objective** To explore the changes and significance of tumor necrosis factor-alpha (TNF-α) in the serum and cerebrospinal fluid (CSF) of children with acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML). **Methods** TNF-α was measured by radioimmunoassay in 31 cases of childhood acute leukemia of various phases. **Results** Serum TNF-α levels pre-therapy in ALL and AML [(24.35 ±4.84) pmol/L, (28.65 ±5.12) pmol/L] were significantly higher than that of control [(11.28 ±1.69) pmol/L], $P < 0.01$. Right after complete remission (CR), TNF-α decreased [(16.42 ±2.57) pmol/L, (14.57 ±3.64) pmol/L] but was higher than that of control ($P < 0.05$). 6, 12, 24, 36 months after CR, serum TNF-α levels in ALL and AML returned to normal. Serum TNF-α increased again and was higher than that of control ($P < 0.01$) when relapsed. CSF TNF-α pre-therapy in ALL and AML [(12.35 ±1.74) pmol/L, (14.56 ±1.92) pmol/L] were also significantly higher than that of control [(7.54 ±0.96) pmol/L] ($P < 0.01$). During CR and continuous CR, CSF TNF-α in ALL and AML patients remained at the level of control ($P > 0.05$). CSF TNF-α level in children with central nervous system leukemia (CNSL) was higher than those without CNSL [(25.62 ±7.14 pmol/L vs (12.15 ±0.89) pmol/L], $P < 0.01$. There was a positive correlation between white blood cell count and TNF-α level in the CSF ($r = 0.942$, $P < 0.05$). CSF TNF-α level decreased gradually after intrathecal therapy, but it decreased more slowly than the white blood cells of CSF. **Conclusions** TNF-α concentration in the serum and CSF may be of great value in reflecting leukemic cell burden, early diagnosis of CNSL and monitoring intrathecal chemotherapy. **[Chin J Contemp Pediatr, 2003, 5(4): 297 - 300]**

**Key words:** Acute leukemia, children; Central nervous system leukemia; Tumor necrosis factor-alpha

## 儿童急性白血病不同治疗阶段血液和脑脊液 TNF-α 变化的研究

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[**摘 要**] **目的** 探讨急性白血病儿童血液和脑脊液中肿瘤坏死因子(TNF-α)的变化及其临床意义。**方法** 采用放射免疫分析法检测 31 例儿童急性白血病治疗前、完全缓解时及连续完全缓解期血液和脑脊液 TNF-α 水平。**结果** 急性淋巴细胞白血病(ALL)和急性髓细胞白血病(AML)治疗前血液 TNF-α 水平[(24.35 ±4.84) pmol/L, (28.65 ±5.12) pmol/L]明显高于正常对照[(11.28 ±1.69) pmol/L]($P < 0.01$);治疗获完全缓解后下降[(16.42 ±2.57) pmol/L, (14.57 ±3.64) pmol/L]但仍高于正常($P < 0.05$);完全缓解后 6,12,24,36 个月稳定在正常水平;但复发时血清 TNF-α 又明显升高,高于对照组($P < 0.01$)。ALL 和 AML 治疗前脑脊液中 TNF-α 水平[(12.35 ±1.74) pmol/L, (14.56 ±1.92) pmol/L]明显高于对照[(7.54 ±0.96) pmol/L], $P < 0.01$;完全缓解后和持续完全缓解期它们与对照组差异无显著性($P > 0.05$)。合并中枢神经系统白血病(CNSL)者脑脊液 TNF-α 明显高于未合并 CNSL 者[(26.47 ±7.14) pmol/L vs (13.15 ±0.92) pmol/L], $P < 0.01$。脑脊液 TNF-α 与脑脊液白细胞数呈正相关($r = 0.942$, $P < 0.05$),经鞘内注射治疗后脑脊液中 TNF-α 逐步恢复正常,但较白细胞恢复慢。**结论** 血液和脑脊液 TNF-α 水平可反映白血病患者的肿瘤负荷及 CNS 的受累程度,是指导治疗的有益指标。

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[**关 键 词**] 急性白血病,儿童;中枢神经系统白血病;肿瘤坏死因子-α

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Tumor necrosis factor-alpha (TNF-α) plays an important role in the regulation of cell growth, proliferation and differentiation. It is involved in the pathogenesis and progress of infectious diseases and tumors. Baxter[1] reported that TNF-α could induce apoptosis or stimulate proliferation of human leukemic cells and that the serum level of TNF-α in leukemia patients was closely related to the treatment effect and prognosis. Kobayashi[2] found that endogenous TNF-α was associated with the sensitiveness to doxorubicin and was a crucial factor of treatment response. We detected the serum and cerebrospinal fluid (CSF) TNF-α level in children with acute leukemia of different phases to explore the clinical significance.

## Subjects and methods

### Subjects

Twenty-one children with acute lymphoblastic leukemia (ALL) aged from 1 $\frac{1}{2}$ to 12 years old, 14 cases were male and 7 female. There were 10 cases of acute myeloid leukemia (AML) aged from 7 to 12 years old, 6 male and 4 female. Five cases of ALL and 1 case of AML complicated with central nervous system leukemia (CNSL) at diagnosis. ALL, AML and CNSL were diagnosed according to the criteria established by national childhood leukemia study group[3]. CSF of control group was obtained from children with acute appendicitis (5 cases) and intussusception (3 cases) before anesthesia. Serum of control group came from 13 healthy children when they received blood examination of trace elements. The age and sex of both control groups were not different from those of study group.

### Collection of samples

Serum and CSF samples were collected before chemotherapy, right after complete remission (CR) and 6, 12, 24, 36 months after CR. CSF of the patients complicated with CNSL was collected before intrathecal treatment. TNF-α in the serum and CSF was detected when the bone marrow relapsed.

### Detection of TNF-α

TNF-α was measured by radioimmunoassay[4].

### Statistical analysis

*t*-test was used to compare TNF-α of different groups. The relationship between TNF-α level and the results of other laboratory indexes of CSF samples was analysed by linear correlation.

## Results

### Serum TNF-α level of various phases

Serum TNF-α levels increased significantly in all ALL and AML patients at diagnosis as compared with the control ($P < 0.01$). However, no significant difference was found in serum TNF-α level between ALL and AML ($P > 0.05$). This elevated serum TNF-α level decreased markedly ($P < 0.05$) but was higher than that of control group ($P < 0.05$) when they obtained CR. 6, 12, 24, 36 months after CR, the serum TNF-α levels in patients with ALL and AML were lower than those of pre-treatment ($P < 0.05$) and were similar to that of control ($P > 0.05$). Serum TNF-α level of relapsed patients increased and was higher than that of control ($P < 0.01$). See Table 1.

### CSF TNF-α level of various phases

Significantly elevated CSF TNF-α level was observed in ALL and AML at diagnosis ($P < 0.01$). Successful chemotherapy resulted in a decrease in TNF-α level, compared with that of pre-treatment ($P < 0.05$). CSF TNF-α level remained lower than that of pre-treatment ($P < 0.05$) and was not statistically different from that of control during continuous CR ($P > 0.05$). It increased and was higher than that of control ($P < 0.01$) when bone marrow relapsed. See Table 2.

### CSF TNF-α level in patients with or without CNSL

CSF TNF-α level in 6 patients with CNSL was higher than that of 25 patients without CNSL [(26.47 ±7.14) pmol/L vs (13.15 ±0.92) pmol/L], $P < 0.01$. It decreased gradually after 4 - 6 times of intrathecal chemotherapy [(9.72 ±1.34) pmol/L]. But the difference of serum TNF-α level between patients with CNSL or without CNSL was not significant [(25.38 ±5.31) pmol/L vs (23.85 ± 4.49) pmol/L], $P > 0.05$.

### Correlation between CSF TNF-α level and other laboratory indexes of CSF

The correlation between CSF TNF-α level and white cell count, protein, sugar, chloride level of CSF was investigated in 6 patients complicated with CNSL. The effects of intrathecal chemotherapy on CSF TNF-α level and other laboratory indexes of CSF were examined. CSF TNF-α level was positively correlated with the white blood cell count of CSF at diagnosis ( $r = 0.942$, $P < 0.05$). Both CSF TNF-α level and white blood cell count decreased gradually after intrathecal injection. However, the CSF TNF-α level decreased more slowly than the white blood cell count and 2 - 3 more additional intrathecal therapy was needed to make the CSF TNF-α return to normal after the normalization of white blood cell count. There was no close relationship between CSF TNF-α level and protein ( $r = 0.252$, $P > 0.05$), sugar ( $r = 0.342$, $P > 0.05$) and chloride level ( $r = 0.302$, $P > 0.05$) at diagnosis.

Table **1** Serum TNF-α in various treatment phases of children with leukemia ( $\bar{x} \pm s$, pmol/L)

| Phases | Control | | ALL | | AML | |
|---|---|---|---|---|---|---|
| | n | TNF-α | n | TNF-α | n | TNF-α |
| Pre-treatment | 13 | 11.28 ±1.69 | 21 | 24.35 ±4.84[a] | 10 | 28.65 ±5.12[a] |
| CR | | | 19 | 16.42 ±2.57[b,c] | 8 | 14.57 ±3.64[b,c] |
| 6 months after CR | | | 19 | 14.58 ±3.42[b] | 8 | 13.53 ±3.58[b] |
| 12 months after CR | | | 17 | 13.62 ±3.28[b] | 7 | 14.75 ±3.12[b] |
| 24 months after CR | | | 15 | 14.51 ±2.86[b] | 5 | 13.19 ±2.17[b] |
| ≥36 months after CR | | | 13 | 12.42 ±2.90[b] | 4 | 13.42 ±2.72[b] |
| Relapse of bone marrow | | | 6 | 22.54 ±6.27[a] | 4 | 25.25 ±7.58[a] |

Note: a vs control $P < 0.01$; b vs pre-treatment $P < 0.05$; c vs control $P < 0.05$

Table **2** Cerebrospinal fluid TNF-α in various treatment phases of children with leukemia ( $\bar{x} \pm s$, pmol/L)

| Phases | Control | | ALL | | AML | |
|---|---|---|---|---|---|---|
| | n | TNF-α | n | TNF-α | n | TNF-α |
| Pre-treatment | 7 | 7.54 ±0.96 | 21 | 12.35 ±1.74[a] | 10 | 14.56 ±1.92[a] |
| CR | | | 19 | 8.63 ±1.32[b] | 8 | 8.94 ±0.33[b] |
| 6 months after CR | | | 19 | 9.44 ±1.36[b] | 8 | 9.13 ±0.82[b] |
| 12 months after CR | | | 17 | 8.78 ±0.96[b] | 7 | 9.63 ±1.15[b] |
| 24 months after CR | | | 15 | 9.23 ±0.83[b] | 5 | 8.71 ±0.68[b] |
| ≥36 months after CR | | | 13 | 9.54 ±1.12[b] | 4 | 9.35 ±1.47[b] |
| Relapse of bone marrow | | | 6 | 8.63 ±0.72[a] | 4 | 10.36 ±1.52[a] |

Note: a vs control $P < 0.01$; b vs pre-treatment $P < 0.05$

## Discussion

TNF-α is a cytokine produced by various cells including T-lymphocyte, monocytes and endothelial cells. Although initially recognized and named for its ability to cause necrotization of tumor masses, recent studies have found that TNF-α produced by carcinocytes from ALL and AML through autocrine stimulates formation of leukemic colony and is involved in pathogenesis and disease progression of leukemia. Camino[5,6] reported that serum TNF-α level increased markedly in patients with acute leukemia and was associated with tumor burden and prognosis. Ferrajoli[7] and other investigators[8,9] found that elevated serum TNF-α level was an independent prognostic factor of various hematological oncology including chronic lymphocytic leukemia, myelodysplastic syndrome and myeloproliferative diseases and was correlated closely with shortened survival and disease deterioration. Our study found that serum TNF-α level in ALL and AML was markedly

elevated before chemotherapy, it decreased gradually right after CR, remained normal during continuous CR but increased at relapse. These indicated that TNF-α was associated with the heavier tumor burden at diagnosis and relapse. So serum TNF-α level may be a useful indicator in determining the efficacy and for monitoring relapse.

We found that TNF-α level in CSF was elevated in children with acute leukemia. This might result from autocrine of TNF-α in the central nervous system or the damage of blood-brain barrier (carcinocytes infiltrating CNS). CSF TNF-α may also be produced by stimulated astrocytes or microglia[10]. Our results suggested that although the clinical and CSF routine examinations were normal, CNS of leukemic children had in fact been infiltrated by carcinocytes at diagnosis. This was consistent with the findings by autopsy that CNS invasion occurred in over 90 % patients with acute leukemia at diagnosis. The decrease of TNF-α after CR also supported this conjecture. CSF TNF-α level was higher in the children with CNSL than those without CNSL. This indicated heavier tumor burden in CNS of former.

We also found that CSF TNF-α level was closely associated with the white blood cell count of CSF. CSF TNF-α level decreased more slowly than the white blood cells did after intrathecal chemotherapy. This is of great significance in judging the therapy effect of CNSL and deciding the course of intrathecal therapy. It is more appropriate that the normalization of TNF-α level in CSF be used as indicator to end intrathecal therapy.

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(Edited by Yan YU)

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(Continued from Page 296)

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