·基础研究·

CD4 细胞内 ATP 浓度代替 CD4/CD8 比值用于评估结核感染患者的免疫状态

刘振杰[1,2]，林东子[3]，涂玉佩[4]，周　丹[4]，李玉美[5]，楼建荣[4]，杨　翔[4]，徐　宁[1,2]

（1. 广州中医药大学第二附属医院检验医学部，广东 广州，510370；2. 广东省中医院检验医学部，广东 广州，510370；3. 佛山市南海区第四人民医院，广东 佛山，528200；4. 广州市雷德生物科技有限公司，广东 广州，510670；5. 东莞市第六人民医院，广东 东莞，523000）

摘　要：【目的】：研究 CD4 T 淋巴细胞内的基础 ATP 浓度代替 CD4/CD8 比值用于评估结核病感染者免疫状态的可行性。【方法】：本研究通过磁珠法分离相同体积全血中的 CD4、CD8 细胞，并计算 CD4 和 CD8 细胞的基础 ATP 浓度来评估结核病感染者的免疫状态，以 QFM（Quantiferon Monitor）检测法作为对照。【结果】：研究发现传统的 CD4/CD8 比值或 CD4 T 细胞计数方法不适用于结核病患者。采用 CD4 细胞的基础 ATP 浓度来监测活动性结核患者和健康人群，在以 290.7×10^{-6} g/L 为 cut-off 值，AUROC 为 0.8719，其灵敏度为 86.21%，特异性为 76.19%，差异有统计学意义（$P<0.000\ 1$）。【结论】：本研究首次使用非外界刺激条件下 $CD4^+$T 淋巴细胞胞内基础 ATP 浓度来评估结核病感染者的免疫状态。提示 $CD4^+$T 淋巴细胞的基础 ATP 浓度比 CD4/CD8 比值可以更好用于评估结核病患者的免疫状态。

关键词：结核分枝杆菌；基础 ATP；CD4 T 淋巴细胞；免疫状态

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Intracellular ATP Concentrations of CD4 Cells Is Better than the CD4/CD8 Ratio for Assessing the Immune Statuses of TB Patients

LIU Zhen-jie[1,2], LIN Dong-zi[3], TU Yu-pei[4], ZHOU Dan[4], LI Yu-mei[5], LOU Jian-rong[4], YANG Xiang[4], XU Ning[1,2]

（1. The Second Affiliated Hospital of Guangzhou University of Chinese Medicine, Guangzhou 510370, China; 2. Guangdong Provincial Hospital of Chinese Medicine, Guangzhou 510370, China; 3. Foshan Fourth People's Hospital, Foshan 528200, China; 4. Leide Biosciences Co., Ltd, Guangzhou 510670, China; 5. Dongguan Sixth People's Hospital, Dongguan 523000, China）

Correspondence to: XU Ning; E-mail: xu_ning21@163.com

Abstract:【Objective】To investigate the feasibility of basal ATP concentration in CD4 T lymphocytes instead of CD4/CD8 ratio in assessing the immune status of tuberculosis-infected patients.【Methods】In this study, the CD4 and CD8 cells in the same volume of whole blood were separated by magnetic beads method, and the basal ATP concentration of CD4 and CD8 cells was calculated to evaluate the immune status of tuberculosis-infected patients. Quantiferon Monitor

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作者简介：刘振杰，硕士，副主任技师，研究方向：生物化学与分子生物学临床检验，E-mail：zjliu1981@126.com；徐宁，通信作者，博士，硕士生导师，E-mail：xu_ning21@163.com

(QFM) assay was used as a control.【Results】Our study confirmed that traditional CD4/CD8 ratio andCD4 T cell counting methodswerenot suitable for TB patients. Using the basal ATP concentration of CD4 cells to monitor active tuberculosis patients and healthy people, when the cut-off value was 290.7×10^{-6} g/L, the sensitivity was 86.21%, and the specificity was 76.19%, and the difference was statistically significant (P<0.000 1).【**Conclussions**】In this study, to the best of our knowledge, we have for the first time used the basal ATP concentration in $CD4^+$ T lymphocytes to evaluate the immune status of tuberculosis-infected patients under non-external stimulation conditions. It is suggested that the basal ATP concentration of $CD4^+$ T lymphocytes can be better used to evaluate the immune status of tuberculosis patients than CD4/CD8 ratio.

Key words: Mycobacterium tuberculosis; basal intracellular ATP; CD4 T lymphocytes; immune status

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2020年世界卫生组织(WHO)全球结核病报告显示，全球有1 000万人患有结核病，150万人死于结核病[1]。世界上约有四分之一的人群感染了结核杆菌，并一直处于潜在的结核感染(LTBI)状态。大约有5%~10%的LTBI会发展成活动性结核(ATB)，其中免疫系统较弱的人有更大的风险。然而，目前没有一种简单且低成本的方法可以用来评估免疫状态[2]。传统研究以每微升CD4 T细胞数量和CD4/CD8比值来评估HIV患者的免疫状态[3]。CD4和CD8 T淋巴细胞是T细胞的重要亚群，在细胞介导的免疫反应中起主要作用。CD4和CD8 T细胞都参与控制结核分枝杆菌感染[4-6]，但对于CD4 T细胞数量或CD4/CD8比值是否可以用来评估结核分枝杆菌感染者的免疫状态尚不清楚。ATP存在于所有生物中，有时被称为细胞能量通货[7]。1983年，Moyer等提出细胞内ATP水平可以反映细胞活性。很多研究[8]提出检测全血中增殖的CD4 T细胞的ATP水平可以反映机体的细胞免疫状态。Cylex还开发了一款商业化的检测试剂(ImmuKnowTM)来检测器官移植患者的免疫功能[9-10]。ImmuKnow™法首先采用PHA刺激CD4细胞，然后分析刺激后CD4细胞中ATP的浓度。但T细胞基础ATP水平能否作为评价结核病患者免疫状态的指标还有待进一步实验。本研究首先通过磁珠法分离相同体积全血中的CD4、CD8细胞，并计算肺结核患者和健康人外周血CD4和CD8细胞的基础ATP浓度，结果发现$CD4^+$ T淋巴细胞的基础ATP浓度比CD4/CD8比值可以更好用于评估结核病患者的免疫状态。现报道如下。

1　材料与方法

1.1　样本及一般资料

肺结核患者62例，年龄35(28~42)岁，来源于东莞市第六人民医院结核科住院的结核病患者。结核阳性包含临床诊断结果(抗体、痰涂片或痰培养阳性等)。健康志愿者42例，年龄34(30~36)岁，来源于为广州军区总医院体检科，结核抗体检测结果为阴性。本研究采用肝素抗凝全血标本，所有样品在检测前都在室温下保存。本研究已通过广东省中医院伦理委员会审查，审查编号：B2017-132。患者和健康对照人群均签署本研究知情同意书。

1.2　流式细胞仪检测CD4 T、CD8 T细胞比率

将50 μL全血分别与20 μL抗FITC-CD3抗体、20 μL抗APC-CD4抗体或20 μL的抗PE-CD8抗体(BD Pharmingen™，美国)混合，室温孵育30 min裂解红细胞，并用PBS洗涤染色的淋巴细胞2次，再用300 μL含1%BSA的PBS重悬。采用流式细胞仪(BD Accuri™ C6)进行分析，以淋巴细胞设门，并用BD CSsamer™分析软件评估CD4和CD8 T细胞的数量(图1)。

1.3　结核病患者和健康人$CD4^+$和$CD8^+$淋巴细胞亚群的分离

用CD4和CD8单克隆抗体(Dynabeads，ThermoFisher公司)包被的磁珠分离纯化CD4 T和CD8 T细胞。首先，将Dynabead(5 μL)洗涤3次，并将其重新悬浮在200 μL的缓冲液A(含0.1%BSA的PBS)中。洗涤后的磁珠10 μL与125 μL抗凝全血混合，室温孵育30 min。为了防止磁珠沉积在试管底部，每15 min轻轻摇晃一次样品。用磁力架分离捕获细胞的磁珠，并去除上清液。洗涤3次后，将

磁珠重新悬浮在125 μL含1% BSA的PBS缓冲液中。

1.4 流式细胞分析法验证分离的CD4和CD8 T细胞亚群

采用流式细胞分析法(FCM)验证从全血中回收的CD4 T和CD8 T细胞的效率。经过细胞分离后,保留已去除CD4 T或CD8 T细胞的血液样本,并以全血样本作为对照。将样品分别加入20 μL抗FITC-CD3抗体、20 μL抗APC-CD4抗体或20 μL抗PE-CD8抗体(BD Pharmingen™,美国),室温孵育30 min。染色完成的淋巴细胞洗涤两次后悬浮在含1%牛血清白蛋白的300 μL PBS中。流式细胞仪(BD Accuri™ C6)进行分析。以淋巴细胞设门,用BD CSsamer™分析软件检测$CD4^+$、$CD8^+$ T细胞亚群。超过98%的CD4和CD8 T细胞从全血样本中成功分离(图2)。

1.5 AO/PI双荧光染色法测定CD4 T、CD8 T细胞活性

在CD4 T和CD8 T细胞悬液10 μL中加入10 μL的AO/PI双荧光染料,孵育2 min后使用自动荧光分析仪(CountstarⓇRigel S2, BD Bioscience,美国)测定细胞存活率。

1.6 健康人和结核病患者CD4、CD8细胞ATP水平测定

用美国Codex Biosciences Co.,Ltd公司的ATP化学发光试剂盒检测纯化的CD4 T细胞和CD8 T细胞中的ATP浓度。在分离和洗涤CD4或CD8 T细胞后,将200 μL裂解液(0.1%Triton X-100)加入到含有Dynabead结合细胞的管中。然后,将50 μL的裂解液与50 μL含荧光素酶的ATP测定溶液混合在1.5 mL的管中。将试管震荡并室温孵育10~15 min,在SpectraMax M3多功能读板仪上读取荧光。ATP标准溶液来自Sigma。在Excel中创建一个标准图形:x轴显示发光强度(相对光单位RLU),y轴显示ATP浓度。由ATP标准曲线可得到良好的线性方程,相关系数R^2=0.999 3。以g/L为单位测定$CD4^+$和$CD8^+$ T细胞产生ATP的浓度。

1.7 相同体积全血和相同细胞数量的CD4和CD8细胞的ATP水平比较

采集39名健康人和43名结核病患者的样本。在等细胞数实验中,纯化后的CD4和CD8 T细胞,分别计数10万个细胞用于ATP水平检测。在等体积实验中,分别取125 μL全血进行CD4、CD8细胞纯化,并进行ATP水平检测。

1.8 健康人和肺结核患者全血经PHA刺激后干扰素-γ的测定

在抗凝管中采集全血标本,每1 mL血细胞加入5 μg PHA进行刺激,经37 ℃孵育20 h后,1 000×g离心5 min,取上清液。用γ-干扰素双抗夹心法检测试剂盒(雷德生物科技有限公司)测定干扰素-γ浓度。

1.9 数据处理和统计分析

本研究中ATP含量和INF-γ含量等计量资料均符合正态分布,以均数±标准差表示。CD4和CD8细胞计数资料不符合正态分布,所以采用中位数M (P_{25}~P_{75})描述。完全随机设计两样本均数的比较采用两样本t检验,P<0.05为差异有统计学意义。ROC曲线绘制及AUC分析采用GraphPad Prism 7软件。

2 结 果

2.1 健康志愿者与肺结核患者CD4/CD8比值分析

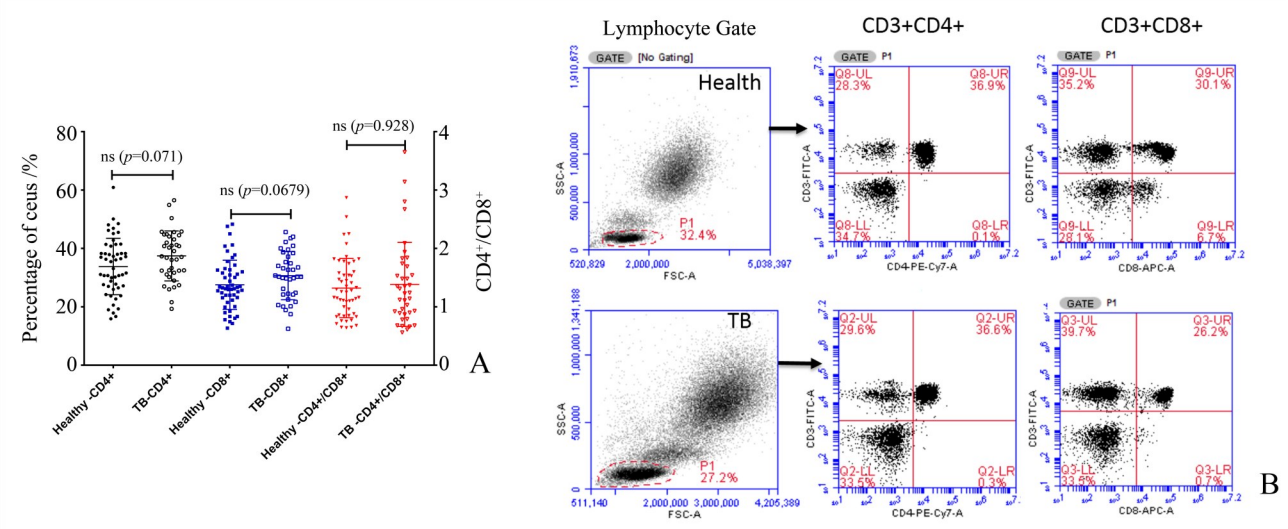
采用流式细胞仪对42例健康志愿者和62例肺结核住院患者的外周血T细胞中CD4和CD8 T细胞的比例进行检测。健康组CD4/CD8的平均值为1.32±0.50。结核组CD4/CD8比值为1.38±0.71。两组CD4/CD8比值的差异无统计学意义(P=0.928 4;图1A)。图1B显示了一名结核病患者和一名健康人的流式细胞仪(FACS)点图分析结果。

2.2 流式细胞仪检测细胞磁珠纯化效果

用包被抗CD4和抗CD8抗体的免疫磁珠对CD4和CD8 T淋巴细胞进行阳性选择。分离的CD4、CD8 T细胞纯度均在95%以上,分选过程对其存活率无明显影响。细胞回收率约为90%(表1)。流式细胞仪检测分离后CD4和CD8 T细胞分别占T细胞总数($CD3^+$)的95.64%和96.88%,说明免疫磁珠法是分离纯化CD4和CD8 T细胞的有效方法。流式细胞仪分析结果表明,细胞的纯度、活性和比例均符合相应要求,能较好地体现真实的细胞比例关系,为后续数据提供了可靠的依据(图2)。

2.3 相同体积全血样本,肺结核住院患者与健康志愿者$CD4^+$和$CD8^+$ T细胞的ATP浓度检测

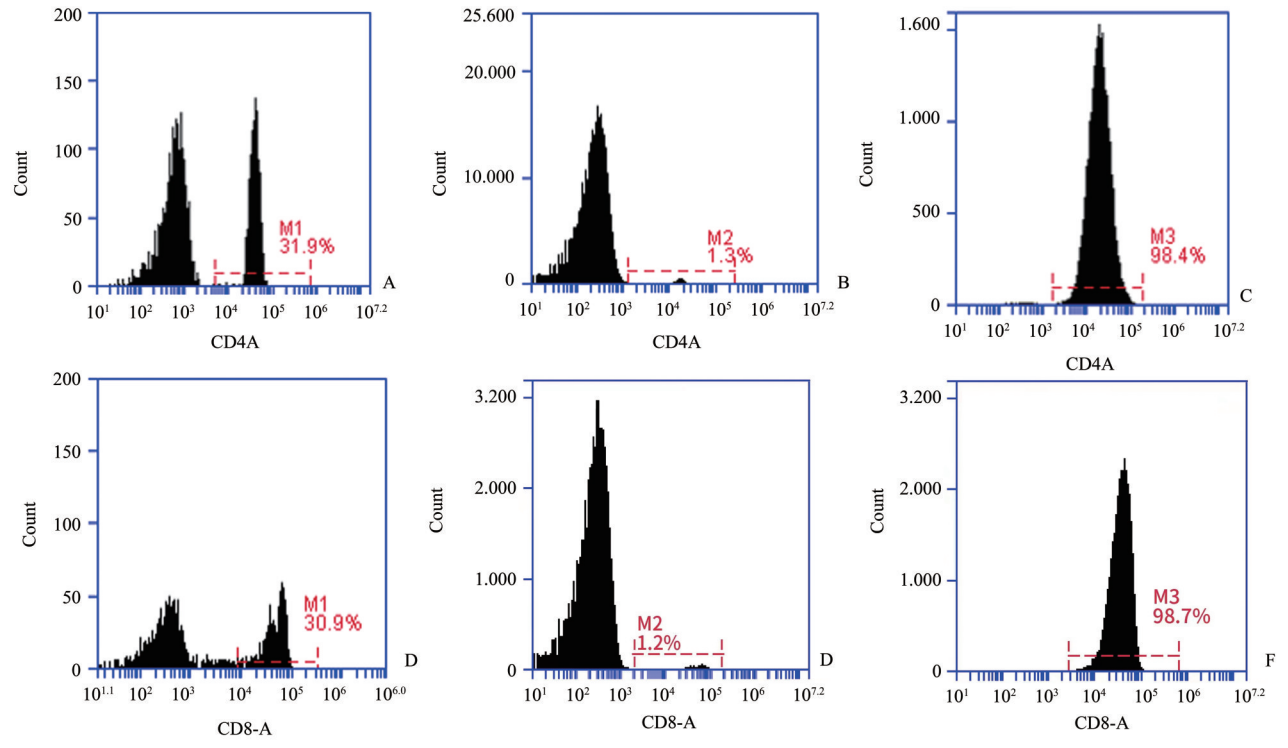
从125 μL的全血中分离CD4、CD8 T细胞,测定ATP水平。在健康样本中,125 μL全血中CD4细胞



The average CD4/CD8 ratio in the healthy group was 1.32±0.50. The average TB group CD4/CD8 ratio was 1.38±0.71. The difference between the TB group and the healthy group was not statistically significant (P=0.928 4；Fig.1A). A FACS dot plot analysis of a TB patient and a healthy person is shown in Fig.1B.

图1　流式细胞术分析42例健康志愿者和62例住院TB患者全血（$CD3^+$）T细胞中$CD4^+$和$CD8^+$T细胞的比例

Fig. 1　The proportions of $CD4^+$ and $CD8^+$ T cells in whole blood T cells（$CD3^+$）harvested from 42 healthy volunteers and 62 TB hospitalized patients analyzed by FCM



$CD4^+$ and $CD8^+$ T cells were separated from 125 μL of whole blood using 20 000 magnetic beads conjugated to anti-CD4 and anti-CD8 in each tube. Cells in the supernatant were analyzed on the BD AccuriTM C6 instrument. Before separation, the percentages of $CD4^+$ and $CD8^+$ cells in the T cell supernatant were approximately 31.9% and 30.9%, respectively (A, D). After Dynabead separation, the percentages of $CD4^+$ and $CD8^+$ cells in the supernatant were reduced to 0.4% and 0.1% (B, F). $CD4^+$ and $CD8^+$ cells were separated completely, with purities of 98.4% and 98.7% (C, F).

图2　磁珠分离前后$CD4^+$和$CD8^+$T细胞的流式细胞术分析

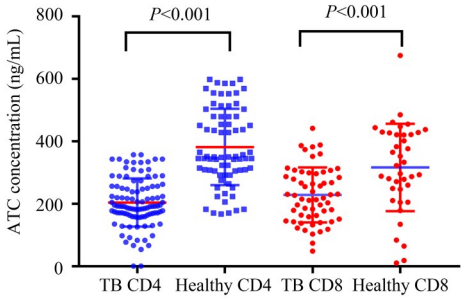
Fig. 2　Flow cytometric analysis of $CD4^+$ and $CD8^+$ T cells before and after Dynabead separation

表1 磁珠分离前后CD4、CD8 T细胞的比较

Table 1 Comparison of CD4 and CD8 T cells before and after magnetic bead separation

Item	CD4		CD8	
	before	after	before	after
Vitality/%	99.36	99.86	99.36	98.40
Purity/%	38.41	95.64	31.82	96.88
Quantity/(*n*/30 μL)	985	907	816	759
Recovery/%	92.55		93.23	

的ATP浓度为349.9(304.8~479.9)×10^{-6}g/L;在肺结核病患者中,125 μL全血中CD4细胞的ATP浓度为192.0(164.7~255.9)×10^{-6}g/L,差异有统计学意义,P<0.001(图3)。健康人125 μL全血的CD8细胞ATP浓度为322.0(246.2~427.3)×10^{-6}g/L,肺结核患者125 μL全血的CD8 ATP浓度为218.2(151.0~296.5)×10^{-6}g/L,差异有统计学意义,P<0.001(图3)。



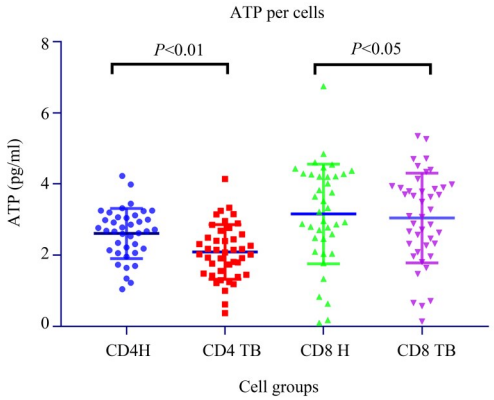
The ATP levels in CD4$^+$ and CD8$^+$ T cells harvested from 125 μL of blood collected from 40 healthy volunteers and 62 TB inpatients were measured. The average ATP levels in CD4$^+$ and CD8$^+$ T cells harvested from healthy people were 349.9 (304.8~479.9) ×10^{-6}g/L and 322.0(246.2~427.3)×10^{-6}g/L, respectively, both of which were higher than those in TB patients (average CD4$^+$ and CD8$^+$ ATP contents of 322.0[192.0(164.7~255.9)×10^{-6}g/L and 218.2(151.0~296.5)×10^{-6}g/L, respectively]. Furthermore, statistically significant differences in the ATP levels in these cell populations were observed between TB patients and healthy individuals (P<0.001).

图3 健康志愿者和结核病住院患者CD4$^+$和CD8$^+$T细胞中ATP的含量

Fig. 3 The ATP contents in CD4$^+$ and CD8$^+$ T cells harvested fromhealthyvolunteers and hospitalized TB patients

2.4 相同细胞数量,肺结核住院患者与健康志愿者CD4$^+$和CD8$^+$ T细胞的ATP浓度检测

健康志愿者单个CD4细胞ATP平均值为(2.62±0.70)×10^{-9}g/L。肺结核患者单个CD4细胞ATP平均值为(2.10±0.77)×10^{-9}g/L,两者差异有统计学意义,P<0.01。健康志愿者单个CD8细胞ATP平均值为(3.16±1.40)×10^{-9}g/L。肺结核患者单个CD8细胞的ATP平均值为(3.05±1.26)×10^{-9}g/L。肺结核患者组CD8 T细胞ATP平均值与健康组差异无统计学意义(图4)。



Samples from 39 healthy people and 42 TB patients were collected. In the equal cell number experiment, 100 000 CD4 cells and 100 000 CD8 cells were separated and counted, their ATP levels were measured. ATP per cell in 2 groups was calculated. In healthy people, the ATP level per CD4 cell average is (2.62±0.70)×10^{-9} g/L. In TB patients, the ATP level per CD4 cell average is (2.10±0.77)×10^{-9} g/L. The difference was statistically significant(P< 0.01). In healthy people, the ATP level per CD8 cell average is (3.16±1.40)×10^{-9} g/L. In TB patients, the ATP level per CD8 cell average is (3.05±1.26)×10^{-9} g/L. The difference was not statistically significant (P> 0.05).

图4 比较TB和健康人群每个CD4和CD8细胞的ATP

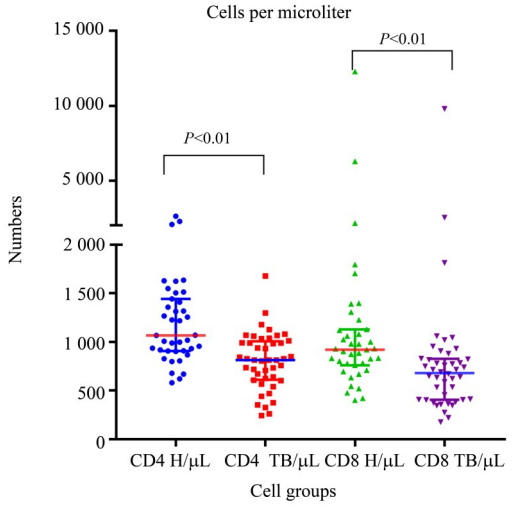
Fig. 4 Comparison of the ATP per CD4 and CD8 cell in TB and health individuals

2.5 健康志愿者和肺结核患者每微升全血中CD4和CD8 T细胞的数量比较

通过检测10万个细胞的ATP水平,可以计算出每个细胞的ATP水平。健康人群每微升血液中CD4 T细胞数为1 067(908~443)个。在结核病患者中,每微升血液中CD4 T细胞中位数为814.5(612.3~1 008),低于健康组,结果有统计学差异,P<0.01。健康人每微升血液中CD8 T细胞数为921(760~1 129)个。结核病组每微升血液中CD8 T细胞682(406.5~827.5),低于健康组。差异有统计学意义,P<0.001(图5)。

2.6 血清γ-干扰素浓度测定

采集50例肺结核住院患者和50例健康志愿者



When the ATP levels of 100000 cells were tested, the ATP levels in each cell could be calculated. In the healthy volunteers group, the median number of CD4 T cells in one microliter of blood is 1 067 (908~443). In TB patients, the median number of CD4 T cells in one microliter of blood is 814.5 (612.3~1008), lower than that in the healthy volunteers group. The difference was statistically significant (P < 0.01).In the healthy volunteers group, the median number of CD8 T cells in one microliter of blood is 921 (760~1 129). In the TB group, the median number of CD8 T cells in one microliter of blood is 682 (406.5~827.5), lower than that in the healthy volunteers group. The difference was statistically significant (P< 0.01).

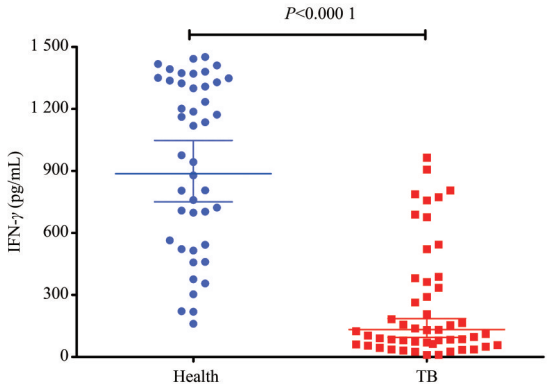
图5　比较健康志愿献血者和结核病患者每微升血液中的CD4和CD8 T细胞数量

Fig. 5　Comparison of the CD4 and CD8 T cell numbers in one microliter of blood in the healthy volunteers and the TB patients

的1 mL全血样本，用非特异性T细胞刺激因子PHA在37 ℃刺激20 h。然后用ELISA法检测50 μL血清中T细胞分泌γ-干扰素的浓度。健康志愿者γ-干扰素平均浓度为1 167.0(664.4~1 374.0)×10^{-9} g/L，明显高于肺结核住院患者的118.0(59.9~367.8)×10^{-9} g/L（图6）。肺结核住院患者的T细胞免疫反应明显弱于健康志愿者，结果显示结核患者缺乏适应性免疫功能。这一结果与从等体积全血中分离的CD4和CD8 T细胞中的ATP含量的检测结果是一致的，如图2所示。实验结果表明，CD4和CD8 T细胞中的ATP水平可以用于反映人体的免疫活性状态。

2.7　正常人和肺结核患者CD4、CD8T细胞基础ATP水平的ROC曲线分析

上述结果表明，健康组与肺结核组等血量的CD4、CD8 T细胞的基础ATP水平差异有统计学意义（P<0.01）。分析了健康志愿者和结核病患者CD4和CD8 T细胞基础ATP浓度的受试者工作特征（ROC）曲线（表2和图7），只有CD4值是敏感和特异的。用Bootstrapping方法确定最优cut-off值。以$CD4^+$细胞ATP浓度为290.7×10^{-6} g/L为cut-off值，AUROC为0.871 9 (0.838 1, 0.937 9)，其灵敏度为86.21%，特异性为76.19%，约登指数为0.624 0。以CD8 ATP浓度为318.10 ×10^{-6} g/L为cut-off值，AUROC为0.716 2(0.607 2, 0.830 5)，其灵敏度和特异性分别为87.93%和51.28%，约登指数为0.374 9。CD4/CD8以1.06为cutoff时，灵敏度39.49%，特异性69.23%，AUROC仅为0.505 8(0.382 0, 0.629 6)，约登指数0.087（图7A）。健康组与肺结核组等血量的CD4、CD8 T细胞数量没有差异，分析了受试者工作特征（ROC）曲线（图7B）。CD4细胞数的AUROC为0.612 1。CD8细胞数的AUROC为0.613 4。



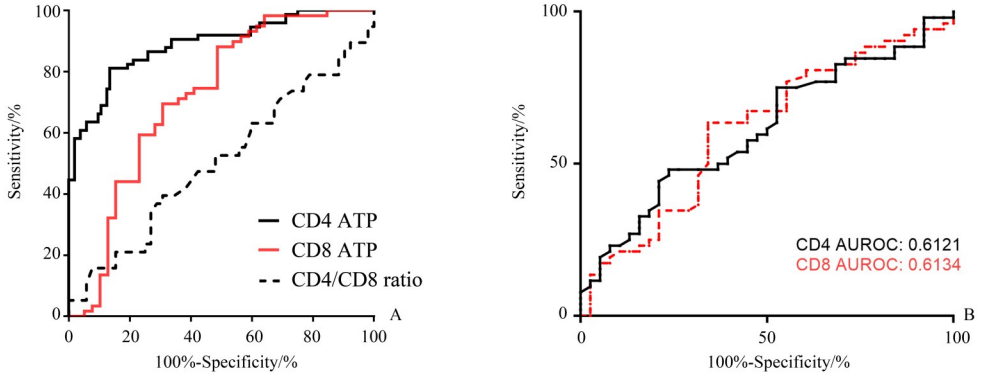
The whole blood samples collected from 50 TB inpatients and 50 healthy volunteers were stimulated with PHA for 20 h, and 50 μL of the supernatant was used to test the concentration of IFN-γ secreted by T cells. In healthy volunteers, the average concentration of IFN-γ secreted by cells was 1 167.0(664.4~1 374.0)×10^{-9} g/L, which was significantly higher than that secreted by TB inpatients 118.0(59.9~367.8)×10^{-9} g/L. The difference of the IFN-γ secreted by TB patients and healthy volunteers was statistically significant (P<0.000 1).

图6　结核患者与健康志愿者PHA刺激20 h后IFN-γ分泌量的比较

Fig. 6　Comparison of the amounts of IFN-γ secreted by TB patients and healthy volunteers after PHA stimulation for 20 hours

3　讨 论

CD4/CD8比值和CD4 T细胞数量用于评估患者的免疫状态，主要应用于HIV患者[3,11]。但在结



A: The receiver operating characteristic (ROC) curve was used to evaluate CD4 ATP, CD8 ATP and CD4/CD8 ratio's ability for classifying TB and Healthy groups. When the cutoff value of $CD4^+$ ATP was set at 290.7 ng/mL, the AUROC was 0.871 9; the sensitivity and specificity were 86.21% and 76.19%, respectively. The Youden index was 0.624 0. When the cutoff value of $CD8^+$ ATP was set at 318.1 ng/mL, the AUROC was 0.716 2; the sensitivity and specificity were 87.93% and 51.28%, respectively. The Youden index was 0.374 9. The CD4/CD8 ratio's AUROC only reached 0.505 8 and the Youden index was 0.087. B: The receiver operating characteristic (ROC) curve of CD4 and CD8 numbers were used to classify TB and Healthy groups. The CD4 number AUROC was 0.612 1. The CD8 number AUROC was 0.613 4.

图7 采用受试者工作特征(ROC)曲线评价CD4 ATP、CD8 ATP和CD4/CD8比值对TB和健康组的进行分类

Fig. 7 Receiver operating characteristic (ROC) curves were used to assess CD4 ATP, CD8 ATP and CD4/CD8 ratio toclassify of TB and healthy groups

表2 CD4和CD8 T细胞基础ATP浓度的受试者工作曲线分析

Table 2 Subject working curve analysis of basal ATP concentration of CD4 and CD8 T cells

Item	cut-off(≥)	Sensitivity/%	Specificity/%	Youden Index	AUROC
CD4 ATP	290.7 ng/mL	86.21	76.19	0.619	0.871 9(0.838 1,0.937 9)
CD8 ATP	318.1 ng/mL	87.93	51.28	0.554	0.716 2(0.607 2, 0.830 5)
CD4/CD8	1.06	39.49	69.23	0.087	0.505 8(0.382 0,0.629 6)

核患者方面,从2010年开始,已有大量的研究证实,结核患者免疫功能下降,但对于免疫力检测的结果有不少相互矛盾的结果。本文采用流式细胞术对结核患者的CD4/CD8比值进行分析,结果显示,肺结核患者的CD4/CD8平均比值与健康志愿者没有差异,健康组CD4/CD8的平均值为1.32±0.50。结核组CD4/CD8比值平均值为1.38±0.71,两组的差异没有统计学意义(P=0.928 4)。因此,不能用CD4/CD8比值来评价结核病患者的免疫状态。进一步研究发现,肺结核患者CD4和CD8 T细胞数量均下降(图5),可以部分解释比值不变的原因。可能从另一方面解释了不同报道中结核病患者免疫状态相互矛盾的结果。本研究首次采用CD4 T淋巴细胞胞内基础ATP浓度来评价结核病患者的免疫状态,比传统CD4/CD8比值或CD4 T细胞数量更能反映患者真正的免疫状态。

ATP存在于所有生物中,有时被称为细胞能量通货[7]。常用来计算细胞繁殖率和细菌数量[8,12-13]。文献报道健康人CD4 T细胞数量在690到2 420个,在HIV患者中,当每微升全血中CD4 T细胞数量低于500个,即意味着免疫缺陷[14]。本研究证实,在健康人群中,每微升全血CD4 T细胞数量均大于500个。但在62例结核患者全血样本中,有5例样本细胞少于500个。肺结核患者组CD4 T细胞数量小于健康组,差异有统计学意义(P<0.01)。

本研究表明,结核病患者的免疫状态处于相对较低水平,可能是由于体内持续的结核分枝杆菌感染,导致结核病患者的T细胞功能下降[15]。在研究慢性病毒感染时,研究人员也发现,抗原在体内的持续存在不仅会降低T细胞的增殖能力,还会降低细胞应答。在很多结核慢性感染的患者中,随着疾病的进展,他们的CD4 T细胞的功能也降低了,导致抗结核免疫保护显著降低[16]。ATP是诱导结核分枝杆菌感染患者单个巨噬细胞凋亡所必需的,适

度的细胞凋亡会降低结核分枝杆菌的活性[8-9,17]。本研究中，结核病患者不仅每微升血液中的CD4 T细胞数量较少，而且其CD4 T细胞中的ATP含量也较少。这两个结果都表明，结核病患者处于免疫状态较低的水平，进一步证实了Moliva的假说[18]。对于CD8 T细胞，本研究尚未发现与结核感染相关的参数。结核病组平均每微升CD8 T细胞数和等体积全血平均ATP水平均低于健康组，但结核组与健康组中每个CD8 T细胞的ATP平均水平并无统计学意义上的差异，具体原因尚不明确。

基础ATP检测方法简单易行。ImmuKnow（IMK）试验结合了细胞刺激、细胞选择和代谢标记物的测定（ATP）来检测细胞免疫状态。PHA刺激后CD4细胞中ATP的含量是衡量淋巴细胞活性的一个指标。本研究在没有PHA刺激的情况下，使用相同体积的血液进行CD4 T细胞分离和ATP检测，从而减少了PHA刺激环节，节省了16 h的孵育时间。文献报道中ImmuKnow检测方法仅用于移植患者免疫力评估，是否适合结核病患者免疫力评估，还有待进一步的实验验证。

QFM是凯杰公司开发的是一种创新的体外诊断测试，可在肝素化全血与先天性和适应性免疫反应兴奋剂孵育后检测干扰素γ（IFNγ），用于评估免疫抑制实体器官移植人群中细胞介导的免疫反应。肺结核患者CD4、CD8 T细胞ATP水平的降低与QFM试验结果一致，提示CD4、CD8 T细胞ATP水平可作为评价结核病患者免疫状态的指标。本研究采用PHA刺激全血细胞，然后检测γ-干扰素分泌水平[19-20]，作为本研究的对照方法。体外培养T淋巴细胞时，外源添加PHA刺激的T细胞，可以诱导CD4 Th1细胞和CD8效应T细胞增殖并分泌γ-IFN[21]。因此，QFM可以作为CD4和CD8 T细胞的联合功能检测。这项研究的结果（如图6所示）表明，住院结核病患者的T细胞免疫反应明显弱于健康人。图3中的数据显示CD4和CD8 T细胞中ATP水平的下降与QFM试验结果也是一致，进一步验证了ATP可以用来评估人体免疫状态。对于CD4 T细胞中ATP水平下降，一个可能的原因是结核感染激活后CD4 T细胞发生凋亡[22-23]，从而导致CD4细胞数量减少；另一种可能是由于长期的结核感染而耗尽了CD4 T细胞[24-25]。这进一步证明了结核病患者的单个CD4 T细胞的ATP含量较低。

CD4 T细胞的ATP水平可作为评价免疫状态的指标。采用ROC曲线分析健康志愿者和结核病患者外周血CD4、CD8 T细胞基础ATP水平，CD4细胞ATP值以290.7×10^{-6} g/L为cut-off值，AUROC最大可达0.8719，其灵敏度和特异性相对较好，超过CD8细胞的ATP。从ROC曲线来看，CD4/CD8的比值在健康人和结核患者中没有区分效果，估计与CD4和CD8的细胞数量在结核患者中同时减少有关。也和CD4/CD8的比值只有在HIV发病时，单一CD4细胞大量减少，此指标较为特异有关，在其他免疫力略微降低的情况下，CD4/CD8并不是一个有效的指标。此推测有待在其他免疫力变化的疾病，如慢性肝炎或风湿类疾病中得到进一步的实验验证。

检测CD4 T细胞的基础ATP水平简单快捷，但是否可以用来评估潜伏结核感染患者的免疫变化，尤其是病情向活动性结核转化中的变化，还有待于进一步研究证实。

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