

Decreased NK cell-mediated cytotoxicity is associated with hypoalbuminemia in hemodialysis patients

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Abstract

End-stage kidney disease (ESKD) and hemodialysis (HD) are a known factor contributing to NK dysfunction. Nonetheless, mechanisms underpinning such dysfunction remain insufficiently elucidated.

We conducted an analysis of NK cell phenotypes across different ages and gender in healthy populations. These parameters and NK cell-mediated cytotoxicity were subsequently compared in HD patients with healthy controls (HC). Finally, the relationship between biochemical data in HD patients with varying levels of NK cell cytotoxicity was studied.

Analysis of NK cell phenotype in 44 individuals reveals specific age-related alterations in NK cell populations and PD-1 expression in healthy individuals. When comparing 30 HD patients with 30 HC individuals, notable changes distinct from aging were found in subtype proportions and surface receptors, indicating renal failure mediates NK dysfunction distinct from normal aging. Functionally, NK cells in HD patients exhibit reduced cytotoxicity. Finally, a significantly higher albumin level was identified among HD patients with higher NK cell cytotoxicity.

This study uncovers phenotypical and functional disparities of NK cells between HD patients and healthy individuals. Impaired NK cell cytotoxicity among HD patients does not completely overlap with immunosenescence and may be attributable to malnutrition, indicated by hypoalbuminemia.

Background

Natural killer (NK) cells are a unique type of immune cell in the human immune system. While derived from common lymphoid progenitors, they are considered a part of the innate immunity system [1, 2]. NK cells lack T cell receptors such as CD3, CD4, and CD8. Instead, they present CD56 and CD16 [3].

NK cells have several important functions. By binding to Fc portion of antibodies and detecting the absence of MHC molecules, they induce ADCC against viral infections and tumor growth [4]. They can also regulate other immune cells through cytokines or direct cytotoxicity [5]. These functions are additionally regulated by specific receptors called killer activation receptors (KARs) and killer-cell immunoglobulin-like receptors (KIRs) [6].

Traditionally, human NK cells can be first divided into two subtypes: CD56^{dim} and CD56^{bright} NK cells. The more abundant CD56^{dim} NK cells progressively increase during aging and are characterized by cytotoxicity and can induce ADCC against tumors or infected cells directly [5, 7]. In contrast, the smaller CD56^{bright} NK cell population serves mainly as cytokine producers and is less cytotoxic [2, 5, 7]. Some studies also found that CD56^{bright} NK cells are the precursors of their CD56^{dim} counterparts [3]. CD56^{dim} NK cells can be further separated into two groups: CD56^{dim}CD16^{bright} NK cells and CD56^{dim}CD16^{dim} NK cells. The majority of CD56^{dim} NK cells were CD56^{dim}CD16^{bright} cells. Their characteristics are similar to CD56^{dim} cells, including the ability to induce ADCC and the inability to produce cytokines [5, 8]. In the

literature, the role of CD56^{dim}CD16^{dim} cells in immunity has been less clear. Most research also considered them as the precursors of CD56^{dim}CD16^{bright} cells, but some suggested that these cells have their own unique function in the immune system [9].

NK cell dysfunction can be observed in many clinical scenarios. Cancer patients have lower NK cell percentages in their blood, and the effector molecules secreted by these NK cells also decrease [10, 11]. Chronic viral infections are also reported to cause impaired INF- γ (a pro-inflammatory cytokine) secretion in adaptive NK cells and lead to NK cell exhaustion [12]. A recent study suggests that stress during cancer surgery induces NK cell suppression, and the “postoperative NK cell dysfunction” may be the reason for cancer recurrence or metastasis [13]. NK cell dysfunction can also appear in patients with the recent pandemic coronavirus disease 2019 (COVID-19), especially in patients with severe complications. This infectious disease has a similar effect on NK cells, causing both “quantity” and “quality” loss of NK cell functions [7, 14, 15].

While less discussed, end-stage kidney disease (ESKD) and hemodialysis (HD) are also considered states of NK cell dysfunction [16, 17]. However, the aspects of NK dysfunction are contradictory in different studies. Some studies found a decrease in the number of total NK cells or NKG2D⁺ NK cells, others found a loss in cytotoxic activity of the patients’ NK cells, still others found a decrease in both aspects [17–21]. Despite the differences, these studies agreed that ESKD and HD lead to a worse immune response from NK cells, causing NK cell dysfunction. Thus, consistent with NK dysfunction, ESKD and HD patients also demonstrate a higher risk of tumor growth or viral infections.

Methods

Aims of study

This study was conducted in light of observations of NK dysfunction in ESKD and conflicting results regarding the precise mechanism of NK cell dysfunction in ESKD. We aimed to investigate the alterations in the NK cell phenotypes in healthy populations of different ages and sexes, compared the alterations in phenotypes and functions between healthy populations and ESKD patients, and identified the factors associated with NK cell dysfunction in ESKD patients.

Human Subjects and Blood Collection

The study included donors from hemodialysis (HD) patients and healthy controls (HC). Donors must be between 20 and 80 years old without infections, recent admission or major surgery in 1 month, cancer treatments including chemotherapy or radiotherapy, pregnancy, or a life expectancy of less than 3 months. After signing the consent form, 10 ml of venous blood samples were obtained from the donors.

Peripheral Blood Mononuclear Cells (PBMCs) Collection and Isolation

PBMCs were isolated from these samples by centrifugation at 3000rpm at room temperature for 20 minutes. Afterward, the cells were double washed and frozen (1×10^7) in liquid nitrogen with fetal bovine serum (FBS) and dimethyl sulfoxide (DMSO) solution until flow cytometry or cytotoxicity assay.

NK Subtype and Receptor Alteration Evaluation

This study used a CytoFLEX flow cytometer (Beckman Coulter). Thawed PBMC cells were stained with the listed conjugated antibodies (Supplementary Table 1). After thawing, cells underwent two washes and then incubated with L/D IR885 at 4°C in the dark for 20 minutes. After that, the cells underwent another two washes and were incubated with the other stains at 4°C in the dark for 15 minutes until analysis. CytExpert for CytoFLEX Acquisition and Analysis Software (Beckman Coulter) and FlowJo™ v10.7.1 (BD Biosciences) were used for data analysis. NK cells were selected, and their subtypes were differentiated based on the expression of CD56 and CD16. The surface receptors of each subtype of NK cells were evaluated respectively. The gating strategy (Supplementary Fig. 1) was adapted from recent studies that acknowledged the importance of CD56^{dim}CD16^{dim} cells in NK populations [8, 9, 22, 23].

NK Cell Cytotoxicity Assay

For determining NK cell cytotoxic function, we adapted the protocol by Kandarian et al. [24]. Thawed PBMCs were rested overnight in the incubator before being co-cultured with harvested K562 target cells labeled with CFSE FITC under E: T ratio 50:1, 25:1, 12.5:1, and 6.25:1, respectively, for 4 hours. Wells with target cell only were used as negative control and K562 cells incubated in 0.1% solution of Tween-20 were used as positive control. The effector cell only condition were also plated to monitor NK cell survival. After 4 hours, all cells were incubated with L/D IR885 at 4°C in the dark for 20 minutes until analysis. CytoFLEX flow cytometer (Beckman Coulter) was used for flow cytometry experiment. CytExpert for CytoFLEX Acquisition and Analysis Software (Beckman Coulter) and FlowJo™ v10.7.1 (BD Biosciences) were used for data analysis.

Ethical Considerations

The Institutional Review Board of Far Eastern Memorial Hospital approved this study (approval project number 103084-E). All participants agreed and signed an informed consent form before the inclusion of this study.

Statistical Analysis

All data were analyzed with the Statistical Package for the Social Sciences (SPSS) software version 22. The alterations in NK cell subtypes and receptors between different age groups and sex, the differences

in cytotoxicity between HD patients and HC, and alterations in the biochemistry data in HD patients with different NK cell cytotoxicity were analyzed using the Mann-Whitney U test. The alterations in NK cell subtypes and receptors between HD patients and HC were analyzed using Student's t-test. To be considered significant, the P-value of the analysis must be less than 0.05.

Results

Analysis of NK subtype and receptor alterations in different age and sex

To understand the general difference in NK cell characteristics among healthy adult populations, we studied the percentages of NK subtypes and their respective surface marker expressions in 44 healthy subjects. The subjects were separated into two groups based on age (cutoff value = 60 years old, Table 1) and sex (Table 2).

Table 1

Comparisons of NK subtype percentages and receptor expression levels of each NK subtype in different age groups

NK% in PBMC	Young group	Elderly group	<i>P</i>
	(n = 13, Age = 49.6 ± 7.3)	(n = 31, 69.6 ± 5.0)	
	9.6 (5.8–13.4)	17.9 (15.4–20.5)	**0.001
CD56 ^{bright} cell% in NK	5.4 (1.0–9.9)	1.6 (1.1–2.1)	**0.004
CD56 ^{dim} cell% in NK	94.5 (90.2–98.7)	98.3 (97.7–98.8)	**0.005
CD16 ^{bright} % in CD56 ^{dim}	89.1 (82.8–95.3)	93.0 (88.8–97.1)	*0.010
CD16 ^{dim} % in CD56 ^{dim}	11.0 (4.7–17.2)	7.0 (2.9–11.2)	*0.010
Surface marker expression of total NK cells			
CD57% in NK	49.4 (38.7–60.1)	68.6 (63.4–73.9)	**0.001
CD226/DNAM-1% in NK	98.8 (98.3–99.3)	99.1 (98.8–99.4)	NS
NKG2D% in NK	84.4 (81.5–87.4)	85.9 (83.0–88.7)	NS
PD-1% in NK	3.2 (-1.1–7.5)	10.8 (6.7–15.0)	**0.001
Surface marker expression of CD56 ^{bright} NK cells			
CD57% in CD56 ^{bright}	0.4 (-0.1–0.8)	3.6 (1.7–5.4)	*0.011
CD226/DNAM-1% in CD56 ^{bright}	99.5 (99.1–100.0)	99.4 (99.0–99.8)	NS
NKG2D% in CD56 ^{bright}	80.7 (71.8–89.6)	85.5 (82.1–88.9)	NS
PD-1% in CD56 ^{bright}	0.4 (-0.5–1.4)	3.8 (1.5–6.1)	**0.006
Surface marker expression of CD56 ^{dim} NK cells			
CD57% in CD56 ^{dim}	49.7 (39.3–60.1)	68.7 (63.4–74.1)	**0.002
CD226/DNAM-1% in CD56 ^{dim}	98.7 (98.2–99.2)	99.1 (98.8–99.4)	NS
NKG2D% in CD56 ^{dim}	85.0 (82.3–87.7)	85.8 (82.9–88.8)	NS
PD-1% in CD56 ^{dim}	3.3 (-1.3–7.8)	11.0 (6.7–15.1)	**0.001

NK cell subset percentages were compared between young versus old group using Mann-Whitney U test. Values are shown as Mean (95% CI); ages of the two subject groups are shown as Mean ± SD. **: p value < 0.01. *: p value < 0.05. NS: non-significant. PBMC: peripheral blood mononuclear cell. DNAM-1: DNAX accessory molecule-1. PD-1: programmed death-1.

NK% in PBMC	Young group (n = 13, Age = 49.6 ± 7.3)	Elderly group (n = 31, 69.6 ± 5.0)	P
	9.6 (5.8–13.4)	17.9 (15.4–20.5)	**0.001
Surface marker expression of CD56 ^{dim} CD16 ^{bright} NK cells			
CD57% in CD56 ^{dim} CD16 ^{bright}	51.1 (41.1–61.1)	68.2 (62.5–74.0)	**0.003
CD226/DNAM-1% in CD56 ^{dim} CD16 ^{bright}	99.2 (98.6–99.7)	99.2 (98.9–99.5)	NS
NKG2D% in CD56 ^{dim} CD16 ^{bright}	84.7 (82.1–87.4)	85.4 (82.3–88.5)	NS
PD-1% in CD56 ^{dim} CD16 ^{bright}	2.9 (-1.7-7.5)	10.5 (6.2–14.7)	**<0.001
Surface marker expression in CD56 ^{dim} CD16 ^{dim} NK cells			
CD57% in CD56 ^{dim} CD16 ^{dim}	28.1 (17.2–39.1)	36.6 (30.1–43.0)	NS
CD226/DNAM-1% in CD56 ^{dim} CD16 ^{dim}	98.7 (90.6–96.9)	96.3 (95.3–97.3)	NS
NKG2D% in CD56 ^{dim} CD16 ^{dim}	90.8 (87.0-94.7)	91.5 (89.1–93.9)	NS
PD-1% in CD56 ^{dim} CD16 ^{dim}	2.4 (-1.0-5.7)	3.3 (1.5-5.0)	*0.018
NK cell subset percentages were compared between young versus old group using Mann-Whitney U test. Values are shown as Mean (95% CI); ages of the two subject groups are shown as Mean ± SD. **: p value < 0.01. *: p value < 0.05. NS: non-significant. PBMC: peripheral blood mononuclear cell. DNAM-1: DNAX accessory molecule-1. PD-1: programmed death-1.			

Table 2

Comparisons of NK subtype percentages and receptor expression levels of each NK subtype in different sex

Age	Male (n = 23)	Female (n = 21)	P
	63.9 ± 11.3	63.5 ± 10.6	NS
NK% in PBMC	16.4 (13.1–19.7)	14.5 (11.0–18.0)	NS
CD56 ^{bright} cell% in NK	1.8 (1.0-2.5)	3.7 (1.0-6.5)	NS
CD56 ^{dim} cell% in NK	98.1 (97.4–98.9)	96.1 (93.4–98.8)	NS
CD16 ^{bright} % in CD56 ^{dim}	92.4 (88.9–95.9)	91.2 (84.9–97.5)	NS
CD16 ^{dim} % in CD56 ^{dim}	7.6 (4.1–11.1)	8.8 (2.5–15.1)	NS
Surface marker expression of total NK			
CD57% in NK	65.8 (58.4–73.2)	59.8 (51.6–68.0)	NS
CD226/DNAM-1% in NK	98.9 (98.5–99.4)	99.1 (98.8–99.4)	NS
NKG2D% in NK	85.7 (82.6–88.8)	85.1 (81.9–88.3)	NS
PD-1% in NK	9.1 (4.7–13.4)	7.1 (1.9–12.4)	NS
Surface marker expression of CD56 ^{bright} NK cells			
CD57% in CD56 ^{bright}	1.7 (0.3-3.0)	3.7 (1.2–6.2)	*0.026
CD226/DNAM-1% in CD56 ^{bright}	99.3 (98.8–99.9)	99.6 (99.3–99.9)	NS
NKG2D% in CD56 ^{bright}	83.1 (77.1–89.1)	85.1 (82.0-88.3)	NS
PD-1% in CD56 ^{bright}	2.7 (0.3–5.2)	2.6 (0.5–4.6)	NS
Surface marker expression of CD56 ^{dim} NK cells			
CD57% in CD56 ^{dim}	64.6 (57.4–71.9)	61.5 (53.0-69.9)	NS
CD226/DNAM-1% in CD56 ^{dim}	98.9 (98.5–99.3)	99.1 (98.7–99.4)	NS
NKG2D% in CD56 ^{dim}	85.9 (82.9–88.9)	85.2 (81.8–88.6)	NS
PD-1% in CD56 ^{dim}	9.2 (4.8–13.7)	7.2 (1.8–12.5)	NS
NK cell subset percentages were compared between male versus female using Mann-Whitney U test. Values are shown as Mean (95% CI); ages of the two subject groups are shown as Mean ± SD. *: p value < 0.05. NS: non-significant. PBMC: peripheral blood mononuclear cell. DNAM-1: DNAX accessory molecule-1. PD-1: programmed death-1.			

Age	Male (n = 23)	Female (n = 21)	P
	63.9 ± 11.3	63.5 ± 10.6	NS
Cell surface receptor expression in CD56 ^{dim} CD16 ^{bright} NK cells			
CD57% in CD56 ^{dim} CD16 ^{bright}	61.8 (54.5–69.0)	64.7 (56.1–73.2)	NS
CD226/DNAM-1% in CD56 ^{dim} CD16 ^{bright}	99.1 (98.7–99.5)	99.3 (99.0–99.6)	NS
NKG2D% in CD56 ^{dim} CD16 ^{bright}	85.8 (82.7–88.8)	84.5 (80.9–88.1)	NS
PD-1% in CD56 ^{dim} CD16 ^{bright}	8.3 (4.0–12.6)	7.3 (1.7–13.0)	NS
Cell surface receptor expression in CD56 ^{dim} CD16 ^{dim} NK cells			
CD57% in CD56 ^{dim} CD16 ^{dim}	34.1 (26.4–41.8)	34.0 (25.3–42.4)	NS
CD226/DNAM-1% in CD56 ^{dim} CD16 ^{dim}	96.2 (94.5–97.9)	94.8 (93.2–96.4)	NS
NKG2D% in CD56 ^{dim} CD16 ^{dim}	90.7 (87.4–94.1)	92.0 (90.0–94.0)	NS
PD-1% in CD56 ^{dim} CD16 ^{dim}	4.3 (1.8–6.9)	1.2 (0.2–2.2)	*0.026
NK cell subset percentages were compared between male versus female using Mann-Whitney U test. Values are shown as Mean (95% CI); ages of the two subject groups are shown as Mean ± SD. *: p value < 0.05. NS: non-significant. PBMC: peripheral blood mononuclear cell. DNAM-1: DNAX accessory molecule-1. PD-1: programmed death-1.			

The percentage of NK cells in PBMC was lower in younger participants than in the elderly group. In addition, young people also had more CD56^{bright} cells and fewer CD56^{dim} cells. Within CD56^{dim} NK cells, we found that there were fewer CD56^{dim}CD16^{bright} cells and more CD56^{dim}CD16^{dim} cells in the young group than in the elderly group (Table 1), confirming the presumed progenitor property of CD56^{dim}CD16^{dim} cells [9].

Consistent with the literature [25–27], CD57 is a cellular senescence marker, and its expressions were higher in total NK cells from the elderly. The difference was statistically significant for all NK cell subtypes except for CD56^{dim}CD16^{dim} cells (Table 1, **36.6%** versus 28.1%).

The expression levels of other investigated surface markers were similar between the two age groups, except for PD-1 expressions in total NK cells and all of their subtypes. In these cell subsets, PD-1 expression levels were increased in the elderly group.

Next, we analyzed the impact of sex on NK cells. The mean age of male and female participants was similar (63.9 ± 11.3 versus 63.5 ± 10.6). We found that the percentage of NK cells in PBMC, distribution of NK cell subtypes, and surface marker expression levels were largely similar between different sexes (Table 2), except for two differences. Firstly, the percentages of CD57⁺ cells in CD56^{bright} NK cells, which

were higher in the female group. Moreover, PD-1 expression in CD56^{dim}CD16^{dim} cells was higher in the male group.

Analysis of NK subtype and receptor alterations between HD patients and HC

To understand if the age-dependent phenotypic changes of NK cells can be observed in hemodialysis patients, we further analyzed the percentages of NK subtypes and their respective surface marker expressions in 30 age-matched HD patients and 30 healthy controls (HC) (mean age $\pm$ SD for HD: 68.9 $\pm$ 9.0 versus HC: 69.8 $\pm$ 5.0, Table 3).

Table 3

Comparisons of NK subtype percentages and receptor expressions of each NK subtype in age-matched hemodialysis (HD) patients versus healthy individuals (HC)

Age	Hemodialysis patients (n = 30)	Healthy controls (n = 30)	P
	68.9 ± 9.0	69.8 ± 5.0	NS
NK% in PBMC	17.4 (14.0-20.8)	18.1 (15.5–20.7)	NS
CD56 ^{bright} cell% in NK	3.2 (1.9–4.5)	1.6 (1.1–2.1)	*0.024
CD56 ^{dim} cell% in NK	96.5 (95.1–97.9)	98.3 (97.7–98.8)	*0.025
CD16 ^{bright} % in CD56 ^{dim}	90.1 (88.1–92.0)	92.9 (88.6–97.2)	NS
CD16 ^{dim} % in CD56 ^{dim}	9.9 (8.0-11.9)	7.1 (2.8–11.4)	NS
Surface marker expression of total NK cells			
CD57% in NK	60.2 (54.0-66.5)	68.0 (62.7–73.3)	NS
CD226/DNAM-1% in NK	99.0 (98.4–99.5)	99.1 (98.8–99.4)	NS
NKG2D% in NK	88.7 (84.0-89.3)	85.9 (83.0-88.9)	NS
PD-1% in NK	13.1 (9.1–17.1)	11.3 (7.0-15.5)	NS
Surface marker expression of CD56 ^{bright} NK cells			
CD57% in CD56 ^{bright}	2.4 (1.3–3.5)	3.0 (1.6–4.3)	NS
CD226/DNAM-1% in CD56 ^{bright}	96.1 (89.4-102.9)	99.4 (98.9–99.8)	NS
NKG2D% in CD56 ^{bright}	79.5 (72.4–86.6)	85.3 (81.8–88.8)	NS
PD-1% in CD56 ^{bright}	3.2 (1.5-5.0)	4.0 (1.6–6.3)	NS
Surface marker expression of CD56 ^{dim} NK cells			
CD57% in CD56 ^{dim}	59.6 (53.1–66.2)	68.1 (62.7–73.5)	*0.045
CD226/DNAM-1% in CD56 ^{dim}	98.9 (98.4–99.5)	99.1 (98.8–99.4)	NS
NKG2D% in CD56 ^{dim}	86.8 (84.2–89.5)	85.9 (82.8–88.9)	NS
PD-1% in CD56 ^{dim}	13.4 (9.4–17.5)	11.4 (7.1–15.6)	NS

NK cell subset percentages were compared between hemodialysis patients versus healthy individuals using Student's t-test. Values are shown as Mean (95% CI); ages of the two subject groups are presented in Mean ± SD. *: p value < 0.05. NS: non-significant. PBMC: peripheral blood mononuclear cell. DNAM-1: DNAX accessory molecule-1. PD-1: programmed death-1.

Age	Hemodialysis patients (n = 30)	Healthy controls (n = 30)	P
	68.9 ± 9.0	69.8 ± 5.0	NS
Cell surface receptor expression in CD56 ^{dim} CD16 ^{bright} NK cells			
CD57% in CD56 ^{dim} CD16 ^{bright}	60.2 (53.6–66.7)	67.6 (61.8–73.4)	NS
CD226/DNAM-1% in CD56 ^{dim} CD16 ^{bright}	99.1 (98.5–99.7)	99.2 (98.9–99.5)	NS
NKG2D% in CD56 ^{dim} CD16 ^{bright}	86.6 (83.7–89.4)	85.4 (82.2–88.7)	NS
PD-1% in CD56 ^{dim} CD16 ^{bright}	12.9 (8.9–16.9)	10.9 (6.6–15.2)	NS
Cell surface receptor expression in CD56 ^{dim} CD16 ^{dim} NK cells			
CD57% in CD56 ^{dim} CD16 ^{dim}	43.9 (35.9–51.9)	35.8 (29.4–42.3)	NS
CD226/DNAM-1% in CD56 ^{dim} CD16 ^{bright}	97.4 (96.4–98.5)	96.6 (95.7–97.4)	NS
NKG2D% in CD56 ^{dim} CD16 ^{bright}	88.7 (85.9–91.5)	91.4 (88.9–93.9)	NS
PD-1% in CD56 ^{dim} CD16 ^{bright}	3.8 (2.4–5.3)	3.8 (1.6–5.1)	NS
NK cell subset percentages were compared between hemodialysis patients versus healthy individuals using Student's t-test. Values are shown as Mean (95% CI); ages of the two subject groups are presented in Mean ± SD. *: p value < 0.05. NS: non-significant. PBMC: peripheral blood mononuclear cell. DNAM-1: DNAX accessory molecule-1. PD-1: programmed death-1.			

The percentage of NK cells in PBMC was similar in the two groups. However, the percentage of CD56^{bright} cells was higher in HD patients than in HC individuals (mean, 95% CI: HD 3.21, 1.91–4.52 vs HC 1.59, 1.05–2.13, $P = 0.024$). On the other hand, the percentage of CD56^{dim} cells in HD patients was lower than in HC (mean, 95% CI: HD 96.53, 95.13–97.93 vs HC 98.26, 97.68–98.84, $P = 0.025$).

Unexpectedly, most surface marker expressions were similar in the two groups, except for the lower percentage of CD57 + cells among CD56^{dim} cells in HD patients than in HC. In summary, age-dependent changes in surface marker expressions and NK cell subset distribution were not observed in hemodialysis patients. In contrast to T cells, which showed prominent premature aging characteristics in hemodialysis patients, NK cells do not exhibit premature aging-related features.

Effects of hemodialysis on NK cell cytotoxicity

Next, we tested the difference in NK cell cytotoxic function by measuring contact-dependent cytotoxicity. NK cell cytotoxicity was analyzed by the ability of NK cells to kill target cells (K562). The experiment was

performed under different effector (PBMC): target cell ratio in 18 HD patients and 17 HC individuals (Fig. 1).

Target cell (K562) death was similar in the two groups without NK cells (Target cell only, mean, 95% CI: HD 2.59, 1.71–3.47 vs HC 2.51, 1.71–3.30, $P = 0.851$).

Target cell death percentages in HD patients were significantly lower than in HC individuals when in different co-culture conditions; including, E: T ratio = 6:1 (mean, 95% CI: HD 3.68, 2.93–4.43 vs HC 5.84, 4.15–7.52, $P = 0.027$), 25:1 (mean, 95% CI: HD 6.33, 4.09–8.56 vs HC 13.22, 7.53–18.91, $P = 0.042$), and 50:1 (mean, 95% CI: HD 10.27, 4.18–16.37 vs HC 19.28, 11.64–26.92, $P = 0.025$). At E: T ratio of 13:1, the difference was borderline statistically significant (mean, 95% CI: HD 4.41, 3.51–5.30 vs HC 8.56, 5.10–12.30, $P = 0.050$).

Association of biochemistry data in hemodialysis patients with impaired NK cell cytotoxicity

Finally, we investigated the clinical parameters associated with an impaired cytotoxicity phenotype in hemodialysis patients. To understand the underlying factors, we split all the hemodialysis patients into two groups based on the K562 death rate in an E: T ratio of 25:1 with a cutoff value of a K562 death rate of 6% (Table 4).

Table 4

Comparisons of clinical parameters and biochemical data in hemodialysis patients with high versus low NK cell cytotoxicity

Age	High cytotoxicity group (n = 7)	Low cytotoxicity group (n = 11)	P
	71.6 ± 9.2	69.3 ± 21.6	NS
Dialysis Vintage (yr)	5.3 (1.1–9.5)	7.4 (3.3–11.4)	NS
BUN (mg/dL)	81.3 (60.2–102.4)	64.4 (55.2–73.6)	NS
Creatinine (mg/dL)	11.0 (9.9–12.1)	10.0 (8.6–11.4)	NS
URR (%)	58.4 (44.4–72.4)	47.3 (39.6–55.0)	NS
Uric acid (mg/dL)	8.0 (6.7–9.4)	7.5 (6.8–8.2)	NS
WBC (10 ³ /μL)	6.8 (5.3–8.4)	5.9 (4.6–7.1)	NS
Hb (g/dL)	11.8 (11.2–12.3)	10.8 (10.1–11.5)	NS
PLT (10 ³ /μL)	193.4 (162.9–224.0)	204.6 (165.8–243.3)	NS
Albumin (g/dL)	4.2 (4.0–4.4)	3.9 (3.7–4.1)	*0.027
Na (mEq/L)	137.1 (135.5–138.8)	124.1 (99.3–148.9)	NS
K (mEq/L)	4.5 (4.0–5.1)	4.4 (4.0–4.9)	NS
Ca (mg/dL)	9.4 (9.2–9.6)	9.0 (8.6–9.4)	*0.027
Corrected Ca (mg/dL)	9.2 (9.0–9.5)	9.1 (8.7–9.5)	NS
P (mg/dL)	5.5 (3.2–7.8)	4.7 (3.9–5.4)	NS
iPTH (pg/mL)	201.9 (99.1–304.8)	278.9 (152.1–405.8)	NS
Ferritin (ng/mL)	336.6 (215.4–457.9)	333.0 (203.1–462.8)	NS
CRP (mg/L)	0.6 (0.2–1.1)	0.3 (0.1–0.4)	NS
Clinical parameters and biochemical data were compared between high versus low cytotoxicity groups in hemodialysis patients using Mann-Whitney U test. Values are shown as Mean (95% CI); ages of the two subject groups are presented in Mean ± SD. *: p value < 0.05. NS: non-significant. BUN: blood urea nitrogen. URR: urea reduction ratio. WBC: white blood cell. Hb: hemoglobin. PLT: platelet. iPTH: intact parathyroid hormone. CRP: C-reactive protein.			

Dialysis vintage, BUN, creatinine, uric acid, urea reduction ratio (URR), white blood cell, hemoglobin, platelet, sodium, potassium, phosphorus, intact-PTH, ferritin, and CRP were not different in the two groups.

Interestingly, among hemodialysis patients, serum albumin level was higher in the high cytotoxicity group than in the low cytotoxicity group (mean, 95% CI: high cytotoxicity group 4.20, 3.97–4.43 vs low

cytotoxicity group 3.93, 3.72–4.14, $P = 0.027$).

In addition, the total serum calcium level was also higher in the high cytotoxicity group patients than in the low cytotoxicity group (mean, 95% CI: high cytotoxicity group 9.39, 9.17–9.60 vs low cytotoxicity group 8.99, 8.59–9.40, $P = 0.027$). However, the corrected serum calcium level was not different in the two groups.

Discussion

This study analyzed the impact of age, sex, and HD on NK subtype and cell surface phenotype alterations. In addition, we investigated the functional difference in NK cell cytotoxicity between healthy individuals and HD patients. We found that HD patients had a lower NK cell cytotoxicity than the general population, but phenotypically, this dysfunctional state does not overlap with the cellular phenotype of aging, hence, immunosenescence. NK cell dysfunction in ESKD is thus a distinct clinical scenario of NK dysfunction. To understand the reason behind the etiology of NK function impairment, we finally looked into the biochemistry data of HD patients and revealed that lower NK cell cytotoxicity was associated with hypoalbuminemia.

The mechanism underlying NK cell dysfunction is complicated. The most discussed is the upregulation of inhibitory receptors, notably NKG2A, TIGIT, and PD-1 [10–12, 14, 28]. Other factors, including downregulation of activating receptors, impaired cell metabolism, and DNA methylation, are also acknowledged [10, 12, 13]. In addition, patients with NK cell dysfunction typically exhibit a lower absolute count of NK cells in the circulation, but analyses on the subtype population of CD56^{dim} and CD56^{bright} NK cells have controversial results. A study discussing NK cell dysfunction in COVID-19 patients states CD56^{dim} NK cells increase in percentage while CD56^{bright} NK cells decrease [15]. While other studies suggest the indifference of subtype percentages or find the “inflamed cell subset” increases with the “proliferation cell subset” decreases [14, 29, 30]. Moreover, impaired NK cells are reported to have a decreased secretion of INF- γ , granzyme B, CD107a, and TNF- α [11, 12, 28]. They are also more likely to go through apoptosis, either by increased expression of apoptosis markers or under the effect of the tumor microenvironment [11, 30].

The percentage of NK cells in PBMC in our study, as well as the percentage of CD56^{dim} cells in NK, were lower in the young group, while CD56^{bright} cells were increased. The results were consistent with the observed changes during normal aging [31–33]. Among CD56^{dim} cells, the CD56^{dim}CD16^{bright} cells are known to increase with age [34], which is also consistent with our results. Furthermore, in our results, the percentages of CD56^{dim}CD16^{dim} cells decreased with aging, which has not been reported in the literature among healthy populations. Because CD56^{dim}CD16^{bright} cells are considered the NK cells with the highest cytotoxic potential, the relative increase in these cells during aging, in combination with decreased NK cell cytotoxicity in the elderly, implies that the control of cytotoxicity is independent of subset differentiation. Previous reports suggested that CD57 expression in NK cells indicates a higher

cytotoxic cell function through differentiation [35]. The increased proportion of CD57⁺ NK cells in the elderly group in our study may explain the preservation of NK cell cytotoxicity during aging [25]. Our data had different results compared to other studies when assessing the difference in CD226 and NKG2D expressions between young and elderly groups. Previous studies found that both CD226 and NKG2D expressions are lower in elderly patients, which is different from our findings that CD226 and NKG2D expressions didn't change with age [27, 36]. The most likely reason is the difference in age selection. The age of participants in our young group was between 30 and 60. In contrast, Phan et al. compared the surface receptor expressions between adults < 30, 30–60, and > 60-years-old. The decrease in NKG2D was shown when comparing the < 30-year-old to the other two age groups. However, there was no difference between the 30-60-year-old group and > 60-year-old group. The results in CD226 in the same study were also similar with the decrease occurring comparing the > 30-year-old group and > 60-year-old group but not the 30-60-year-old group and > 60-year-old group [27]. One study investigated PD-1 expression on NK cells and found that among older people, PD-1⁺ cell percentage is higher in total NK cells and CD56^{dim} cells while non-significant in CD56^{bright} cells [37]. Our study demonstrates similar results with an increase in PD-1⁺ cells in total NK cells and all their subtypes during aging.

In the literature, it was reported that men have a higher percentage and a higher absolute number of NK cells than women, as well as increased percentages of CD56^{dim} cells and CD57 expressions, while NK cells in women have a higher cytotoxicity [27, 38, 39]. Our results, while not reaching significance, yielded a similar pattern with higher percentages of NK cells, CD56^{dim} cells, and percentages of CD57⁺ cells in men. Interestingly, in our results, the percentages of CD57⁺ cells in CD56^{bright} cells were higher in women than in men. This may help explain NK cells' more potent cytotoxic functions in women. When it comes to the effect of sex on surface marker expressions, NKG2D is the most discussed. Some studies found that estradiol induces NKG2D ligand expression, which is associated with a decrease in NKG2D expression [40, 41]. However, one study that directly compares NKG2D expressions between men and women concluded that NKG2D expression levels were not different within total NK cells and CD56^{dim} cells, while men had higher NKG2D expression within CD56^{bright} cells [27]. Our results partially align with this study, with the main difference that our NKG2D expressions did not differ between sexes. No study, to the best of our knowledge, has investigated the effect of sexes on PD-1 expression among NK cells. Our results found an increase of PD-1⁺ cells in CD56^{dim}CD16^{dim} cells among men. The finding aligns with previous studies that NK cells in men have a lower cytotoxicity.

ESKD and HD are known to be associated with NK cell dysfunction [16, 17]. The higher percentages of CD56^{bright} cells and the lower percentages of CD56^{dim} cells, along with a decrease of CD57 expression in CD56^{dim} cells, which we found in this study, support the impaired cytotoxicity in HD patients. Interestingly, while some references suggest that NKG2D expression may be lower in HD patients [16, 18, 42], we did not detect differences in NKG2D expression between HD and HC groups, similar to a previous study [17]. We also did not detect a significant increase in PD-1 expression among HD patients, as shown by a previous study [43]. Combining the results of phenotypic and functional analyses, we

demonstrate that despite functional impairment of NK cells in HD patients, the similar expression levels of NKG2D and PD-1 suggest a potential distinct mechanism of NK cell dysfunction in ESKD.

Nutritional deficiencies—particularly of micronutrients such as zinc and vitamin D, as well as overall macronutrient insufficiency—are consistently associated with impaired NK cell cytotoxicity [44]. Mechanistically, insufficient protein and energy availability may restrict amino-acid supply, bioenergetic capacity, and mTOR-dependent effector programming, leading to impaired NK-cell proliferation, degranulation, perforin/granzyme-mediated killing, and IFN- γ production [45]. HD patients frequently develop protein–energy wasting (PEW), a form of malnutrition, with reported prevalence as high as 70%. Nutritional depletion in HD patients with PEW may compromise NK cells by restricting amino-acid and energy availability required for mTOR-dependent cytotoxic programming, thus contributing to premature mortality in these patients [46]. Calcium (Ca) also has many roles in human cell function. Immune cells heavily depend on Ca receptors and channels in cell activation against pathogens and regulation to prevent autoimmunity [47, 48]. Several references suggest that hypocalcemia may have caused immune dysfunction, including adverse outcomes in patients with COVID-19 and higher systemic lupus erythematosus activity [49, 50]. Our results also found a decrease in serum Ca levels in HD patients within the low cytotoxicity group. However, this finding might result from the difference in serum albumin level, as the difference of corrected serum Ca level showed no difference.

The study had several limitations. The number of participants we analyzed was small, and the population came from one medical center. In addition, underlying medical issues such as tumors or chronic viral infections, which may also cause NK cell dysfunction, were ignored. Future studies with larger populations and multiple medical institutions with confounders in each participant's medical history may also be conducted.

Conclusion

This is the first study to analyze the relationship between nutritional depletion, indicated by serum albumin level, and NK cell dysfunction in HD patients. The findings have direct implications for clinical practice and apply to improving NK cell functions in these populations. The results support adding nutrition or direct albumin supplements in HD patients, which may prevent complications related to NK cell dysfunctions, such as failure to inhibit tumor growth or severe complications when undergoing infections. Further research may be conducted to confirm this theory, such as functional changes in NK cell cytotoxicity when HD patients are treated with nutrition or albumin supplements.

Abbreviations

ESKD: end-stage kidney disease. HD: hemodialysis. HC: healthy controls. PBMC: peripheral blood mononuclear cell. DNAM-1: DNAX accessory molecule-1. PD-1: programmed death-1

Declarations

Ethics Approval and Consent to Participate

The study was approved by the FEMH's institutional ethical committee (FEMH 103084-E). The study was carried out according to the ethical standards of FEMH. All human subjects provided informed consent.

Consent for Publication

Not applicable

Competing interests

The authors declare no competing interests related to this study.

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Author Contribution

Bing-Rung Chuang and Yen-Ling Chiu designed the study. Yen-Ling Chiu and Yu-Sen Peng provided the funding source; Yen-Ling Chiu, Kai-Hsiang Shu, Ju-Yeh Yang, and Yun-Ting Juan recruited study participants; Bing-Rung Chuang, Kai-Hsiang Shu, Yun-Ting Juan, I-Yu Chen, and Yen-Ling Chiu performed tests on subjects, processed the data, performed experiments, and analyzed the results; all authors reviewed and edited the manuscript; Bing-Rung Chuang and Yen-Ling Chiu wrote the draft of this manuscript and submitted the manuscript.

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Data Availability

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy.

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Figures

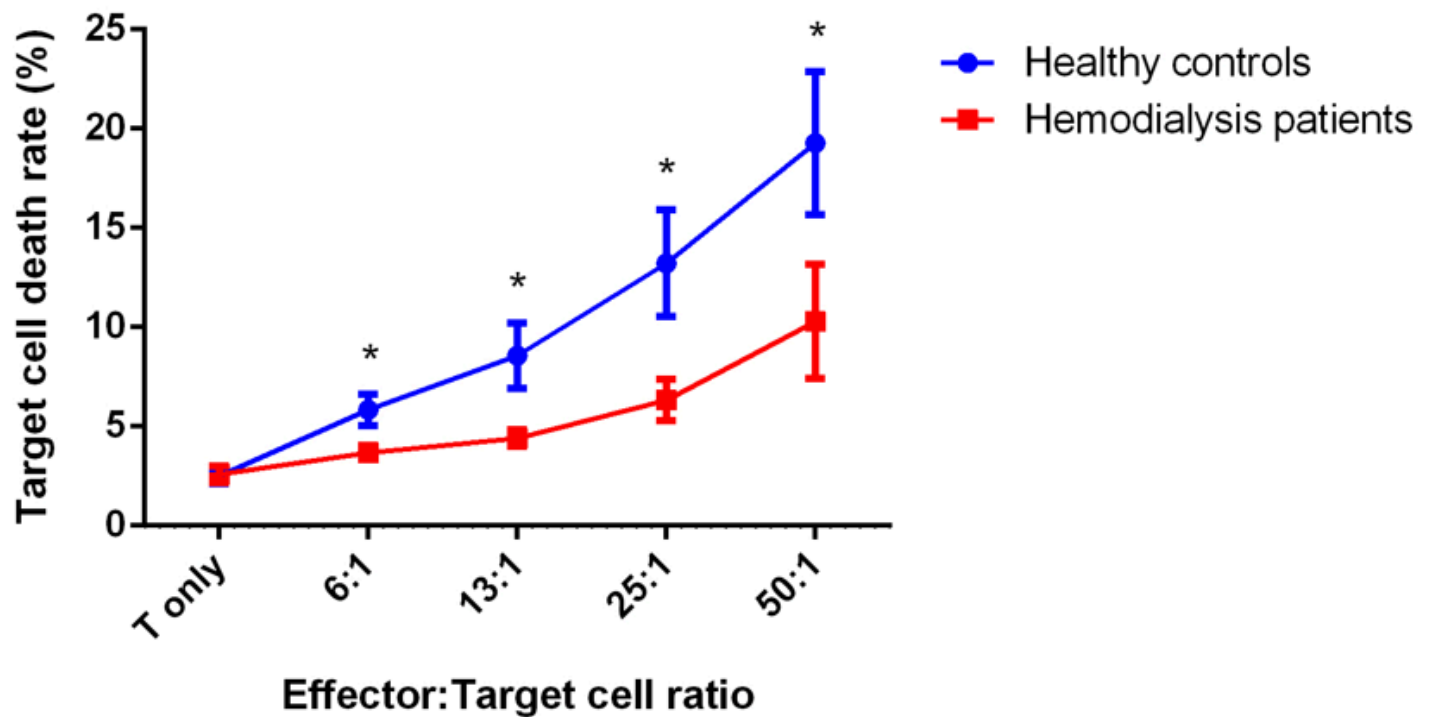


Figure 1

Impaired NK cell-mediated cytotoxicity in hemodialysis patients

Target cell (K562 cell line) death rates were compared between hemodialysis patients versus healthy individuals using Mann-Whitney U test. Graphs are shown as Mean ± SEM. *: p value < 0.05. T: target cell

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