

Heterophile antibody interference associated with natural killer cell therapy

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Abstract. The adoptive transfer of *ex vivo*-expanded natural killer (NK) cells has recently been employed as an alternative cancer treatment in certain institutions. However, the safety profiles of this strategy remain uncharacterized. We evaluated three patients who exhibited elevated serum parathyroid hormone (PTH) levels without the relevant clinical manifestations and had a history of autologous NK cell therapy. The serum PTH concentration was measured using a second-generation PTH assay, and the serum thyroglobulin concentration was measured using a second-generation thyroglobulin assay. Subsequently, the PTH or thyroglobulin concentration obtained using heterophile-blocking tube (HBT) for a secondary confirmation assay was measured and compared with the result of the initial assay. The three patients had falsely elevated serum PTH and thyroglobulin levels owing to heterophile antibody interference associated with NK cell therapy that persisted for at least up to 12 months after the treatment and was confirmed by normalization of hormone levels after HBT treatment. We propose that certain types of mouse monoclonal antibodies used to stimulate NK cells can induce heterophile antibodies. Abnormal laboratory test results in individuals administered NK cell therapy without the relevant clinical manifestations must be examined in the context of heterophile antibody interference to avoid misdiagnosis and unnecessary testing.

Key words: Heterophile antibody, Natural killer cell, Parathyroid hormone, Thyroglobulin

CANCER IMMUNOTHERAPY, which works by activating the body's immune system, has become an increasingly important treatment option for cancers [1]. In recent years, it has emerged as a significant area of fundamental research in cancer immunology [2]. There are many immunotherapeutic approaches, such as immune checkpoint blockade and T cell therapy.

Natural killer (NK) cells play a key role in innate immunity by destroying a variety of abnormal or stressed cells [3]. Distinct from the recognition of other lymphocytes, NK cell recognition is not controlled by antigen specificity but rather by integrated signals from activating and inhibitory receptors, which are recruited by ligands expressed on the surface of putative target cells. NK cell-mediated immunotherapy is one of the treatment

options for cancer that has attracted increased attention in recent years. Its efficacy can be enhanced by immune stimulants, such as cytokines and antibodies, and the adoptive transfer of activated *ex vivo*-expanded NK cells [1].

In the treatment of solid tumors, the adoptive transfer of *ex vivo*-expanded NK cells has been attempted as a novel therapeutic option [4]. This therapy involves isolating NK cells from the peripheral blood of a patient, culturing them to expand the number of cells in a specific medium, and finally transfusing the expanded cells back into the patient's body [5]. It is a direct and fundamental approach for replacing, restoring, and improving immune system function. A previous study determined that NK cell therapy can be considered a safe alternative to standard cancer treatment [4]. However, despite its potential, solid evidence of the efficacy of this treatment for cancer is lacking, and data regarding the safety of NK cell therapy in humans are limited [6].

Here, we report false-positive heterophile antibody interference after autologous NK cell therapy. To our knowledge, it is the first report indicating that autologous NK cell therapy can generate heterophile antibodies and result in false laboratory test outcomes.

Submitted Jun. 9, 2020; Accepted Jul. 3, 2020 as EJ20-0349

Released online in J-STAGE as advance publication Jul. 22, 2020

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Material and Methods

We evaluated three patients who exhibited elevated serum parathyroid hormone (PTH) levels without the relevant clinical manifestations and had a history of autologous NK cell therapy. This study was approved by the Institutional Review Board of Severance Hospital, Yonsei University Health System, Seoul, Korea (No. 4-2020-0072), and informed consent was obtained from the patients.

The serum PTH concentration was measured at our hospital using a second-generation PTH assay (Elecys PTH; Roche Diagnostics, Mannheim, Germany) on the Cobas e801 immunoassay analyzer (Roche Diagnostics). The serum thyroglobulin concentration was measured using a second-generation thyroglobulin assay (Elecys Tg II; Roche Diagnostics) on the Cobas e801 immunoassay analyzer.

Heterophile-blocking tube (HBT; Scantibodies Laboratory, Santee, CA, USA) was used for a secondary confirmation assay whose results were compared with those of the first assay according to the manufacturer's instructions. In total, 500 μ L of the sample was pipetted into a tube, mixed with lyophilized pellets by inverting the tube several times, and incubated for 1 h at room temperature. Subsequently, the PTH or thyroglobulin concentration in the HBT-treated sample was measured again for comparison with the initial result.

Results

The first patient was a man in his late 40s who was referred to our hospital for suspected thyroid cancer recurrence. He had a history of papillary thyroid cancer, which was treated by total thyroidectomy in August 2010, followed by 100 mCi of radioactive iodine in April 2011. A subsequent I-131 whole-body scan revealed no persisting cancer or evidence of recurrence. His thyroglobulin level was <0.2 ng/mL [triiodothyronine (T3) 0.68 ng/mL, free thyroxine (T4) 1.11 ng/dL, thyroid-stimulating hormone (TSH) 3.41 μ IU/mL, thyroglobulin antibody 2.46 IU/mL] in December 2017. However, the thyroglobulin level increased to 37.91 ng/mL in December 2018 (T3 0.91 ng/mL, free T4 1.13 ng/dL, TSH 10.08 μ IU/mL, thyroglobulin antibody 0.98 IU/mL). A neck ultrasound did not reveal a likely source for the detectable thyroglobulin. Fluorodeoxyglucose positron emission tomography-computed tomography showed no pathological uptake.

The thyroglobulin level in the patient was measured at our hospital, and found to be 1.9 ng/mL (T3 0.69 ng/mL, free T4 1.07 ng/dL, TSH 11.22 μ IU/mL, thyroglobulin antibody <10 IU/mL), whereas an elevated PTH level

(1,649.0 pg/mL) was detected following a routine blood test with normal serum calcium levels (9.4 mg/dL). The 25-OH-vitamin D level was 19.97 ng/mL, and 24-h urine calcium was 86.5 mg. As the repeat measurement confirmed an abnormal PTH (1,526.0 pg/mL) value, 99mTc-methoxyisobutylisonitrile (MIBI) single-photon emission computed tomography/computed tomography scintigraphy (SPECT) was performed, which showed no abnormal findings. A targeted next-generation sequencing panel test (for 400 genes associated with hereditary endocrine disorder including parathyroid disease, such as *CASR* and *CDC73*) only detected some variants of unknown significance. The levothyroxine dose was increased from 150 to 175 mcg, and 25-OH-vitamin D replacement was started. The PTH level gradually decreased during the follow-up (736.8 pg/mL in April 2019 and 318.0 pg/mL in September 2019) (Fig. 1A). The thyroglobulin level also decreased slightly (1.7 ng/mL in April 2019 and 0.7 ng/mL in September 2019). The 25-OH-vitamin D level was 27.78 ng/mL in September 2019. At that point, the patient revealed that he had undergone NK cell therapy from January 2017 to October 2018.

The second patient was a woman in her late 60s who was diagnosed with papillary thyroid cancer and underwent a right thyroidectomy in May 2009. She was receiving levothyroxine (50 mcg) with no persisting cancer or evidence of recurrence. Her PTH level gradually increased from 2017 onward with normal serum calcium; thus, she was referred to the endocrinology department (Fig. 1B). Her PTH level was 4,667.0 pg/mL with normal serum calcium (9.2 mg/dL) and 25-OH-vitamin D (58.16 ng/mL) in September 2019. The 24-h urine calcium value was 289.8 mg. Although 99mTc-MIBI SPECT was performed, no abnormal uptake was observed. The patient declared that she was administered 25-OH-vitamin D by injection and had undergone NK cell therapy from February 2017 to August 2019.

Notably, these two patients underwent identical autologous NK cell therapy procedures at the same hospital. Our PTH immunoassay relies on the use of monoclonal anti-PTH antibodies of mouse origin. It is known that human antibodies directed against mouse immunoglobulins (human anti-mouse antibody), which are the most commonly encountered heterophile antibodies, can interfere with immunoassays and produce spurious results. We considered that the abnormal laboratory test results obtained for our patients may have been owing to heterophile antibody interference associated with NK cell therapy. We searched for their NK cell therapy methods using their registered patent (WO2016209021A1) and found that they use not only interleukin-2 but also combinations of cytokines and the anti-NKp46 antibody

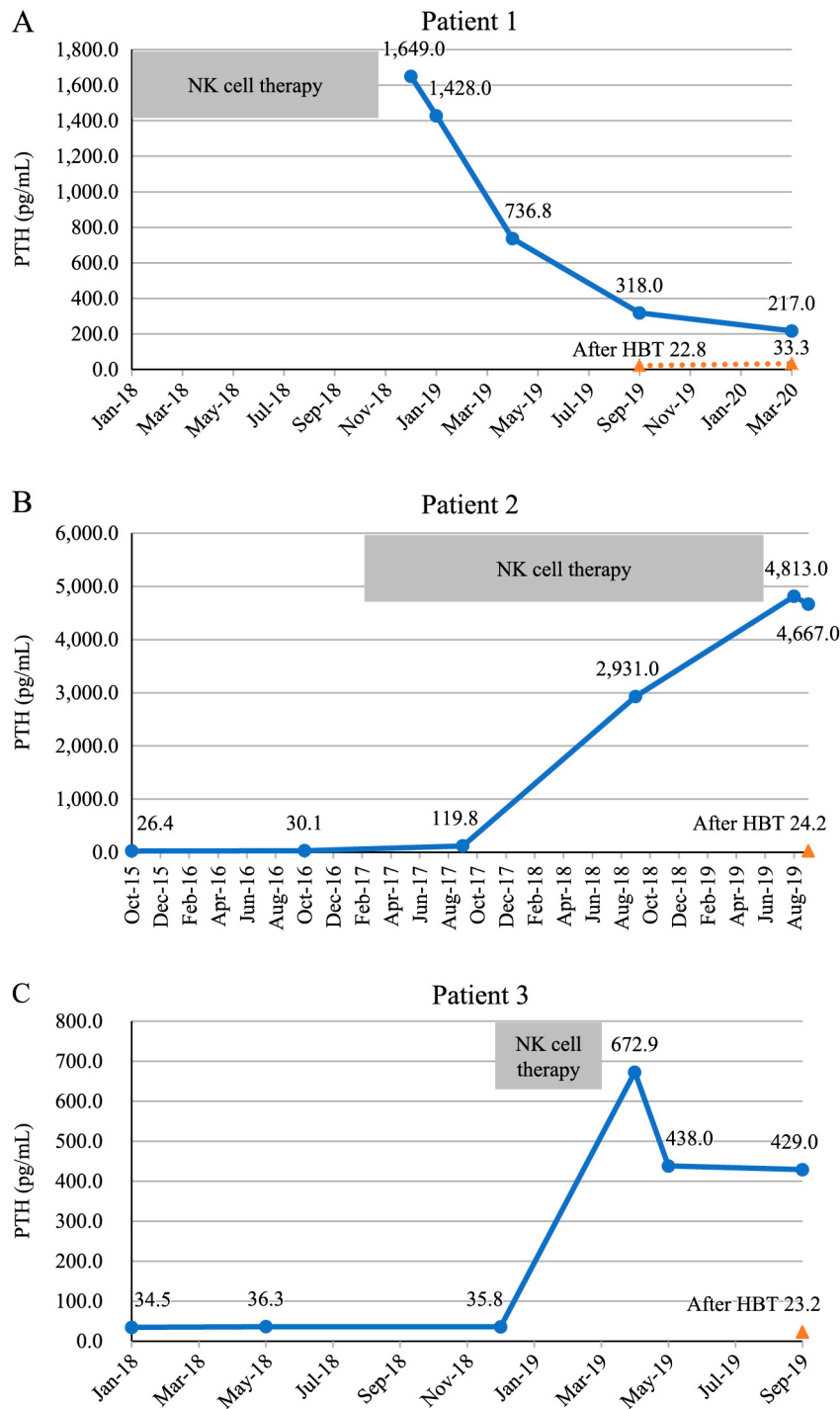


Fig. 1 Parathyroid hormone (PTH) levels [circle dots] of patients over time. Data are shown for patient 1 (A), patient 2 (B), and patient 3 (C). The PTH levels after heterophile-blocking tube (HBT) treatment are also indicated [triangle dots]. The duration of natural killer (NK) cell therapy is indicated by the gray box.

to stimulate *ex vivo* expansion and NK cell activation. We also found that other groups use similar antibodies as immune stimulants. The anti-NKp46 antibody is obtained from mice and is a potential factor in inducing heterophile antibodies by the contamination of the infused expanded NK cells. Rheumatoid factors can also act as heterophilic antibodies. However, the rheumatoid

factor level was found to be 18.0 IU/mL in the first patient and 9.0 IU/mL in the second patient.

To test the effect of heterophile antibodies, we treated the remaining samples, stored at 4°C within 1 week after the initial PTH measurement, with HBT. HBT treatment resulted in the PTH level being returned to normal in both of our patients; thus, the presence of a heterophile

Table 1 Laboratory analyses before and after HBT treatment

Patients	Before HBT	After HBT
Patient 1		
PTH	318.0 pg/mL	22.8 pg/mL
T3	0.92 ng/mL	0.94 ng/mL
Free T4	1.11 ng/dL	1.20 ng/dL
TSH	1.38 μ IU/mL	1.52 μ IU/mL
Thyroglobulin	0.7 ng/mL	<0.1 ng/mL
Thyroglobulin Ab	<10 IU/mL	<10 IU/mL
Patient 2		
PTH	4,667.0 pg/mL	24.2 pg/mL
Patient 3		
PTH	429.0 pg/mL	23.2 pg/mL

HBT, heterophile-blocking tube; PTH, parathyroid hormone; T3, triiodothyronine; T4, thyroxine; TSH, thyroid-stimulating hormone. Reference range: PTH 15–65 pg/mL, T3 0.61–1.16 ng/mL, Free T4 0.80–1.23 ng/dL, TSH 0.41–4.30 μ IU/mL, Thyroglobulin 0–33.2 ng/mL, Thyroglobulin Ab 0–130.6 IU/mL.

substance in the serum sample was indicated (Table 1). The thyroglobulin level was less than 0.1 ng/mL after HBT treatment in the first patient. The serum thyroglobulin concentration was measured in a manner similar to that used for measuring PTH. This assay is also a sandwich immunoassay that uses monoclonal antibodies of mouse origin and is subject to heterophilic interference.

The third patient was a woman in her early 70s with low bone mass who visited our endocrine unit for a routine health assessment. She was administered 25-OH-vitamin D supplementation. Her PTH (672.9 pg/mL) level suddenly increased, whereas her serum calcium (9.1 mg/dL) and 25-OH-vitamin D (31.46 ng/mL) levels were normal in April 2019. No significant MIBI uptake suggestive of parathyroid adenoma or hyperplasia was observed during 99mTc-MIBI SPECT. However, a high PTH level was maintained with normal calcium levels (Fig. 1C). The patient was administered NK cell therapy at the same hospital as the above two patients from December 2018 to February 2019 because of health concerns. Her remaining samples, stored at 4°C within 1 week after the initial PTH measurement, were treated with HBT, which resulted in her PTH levels returning to normal (Table 1). Her rheumatoid factor level was also in the normal range (4.3 IU/mL).

Discussion

Heterophile antibodies are endogenous antibodies that bind to the reagent antibody [7]. They can be produced in response to external animal antigens or as anti-self-

antibodies, such as rheumatoid factors, which have cross-reactivity with the reagent antibody. These antibodies can cause significant interference in any immunoassay, and “sandwich” immunoassays are particularly susceptible to this interference. When heterophile antibody interferences occur, they typically result in false-positive test results [8]. Heterophile antibody interference is not widely recognized by laboratorians or clinicians. Additionally, detecting this interference in the clinic is difficult. The best option is to repeat the test using a different type of assay or heterophile-blocking reagents [9]. Although clinically significant interference by heterophile antibodies in immunoassays is rare, it can pose a problem for patients treated with drugs containing non-human antibodies such as OKT3, a murine monoclonal IgG21 antibody [10, 11].

The number and cytotoxicity of NK cells, which enhance the host immune responses against cancer, are often low in patients with cancer [12]. Therefore, researchers developed a large-scale *ex vivo* NK cell expansion method and introduced autologous NK cell therapy [12]. Furthermore, researchers attempted to assess the safety of autologous immune cell therapy for treating cancers [5]. However, the NK cell-based immunotherapy of solid cancers remains controversial [6]. In this study, we report false-positive heterophile antibody interferences after autologous NK cell therapy for the first time. We realized that immune stimulants, such as cytokines and antibodies, used in *ex vivo* NK cell expansion might aid us in finding the reasons underlying heterophile antibody interferences. Using a commercially available HBT to reduce interference, we demonstrated that murine monoclonal antibodies, which may result in heterophile antibody induction, cause false abnormal laboratory test results. There are some previous studies on heterophile antibody interferences of unknown origin [7, 13–15]. However, the limitations associated with new treatment modalities are critical issues in the clinical field.

Although the therapeutic applications of NK cells have been widely studied in the last few decades, there is still a considerable knowledge gap that needs to be bridged before translating this approach into the clinic [6]. However, some patients with cancer have unquestionable faith in new therapies and are likely to receive NK cell therapy. Certain ordinary people unaffected by disease, such as our third patient, receive NK cell therapy to enhance their immunity. We cannot comprehensively comment on the efficacy of autologous NK cell therapy in the present study, but what we can comment on is that NK cell therapy-related heterophile antibody interference is problematic; it is of particular concern because most antibodies used in immunoassays are

derived from mice [8].

Autologous NK cell therapy is not an antibody treatment. However, a murine monoclonal antibody used for *ex vivo* NK cell expansion may act as a foreign substance in patients and create a new set of antibodies that react with immunoglobins of murine origin, namely human anti-mouse antibodies (HAMAs). These antibodies can cause not only heterophile antibody interference but also a HAMA response. The HAMA response, an immune response against the foreign protein (mouse antibody), is an allergic reaction that can range from mild, such as a rash, to more extreme and life-threatening responses, such as renal failure [16]. Although no allergic reactions were observed in our patients, precautions should be taken because autologous NK cell infusion is usually carried out in a multicycle protocol, which can increase adverse immune responses.

If autologous NK therapy is effective, safer approaches are required to stimulate the *ex vivo* expansion and activation of NK cells. Attenuated or non-immunogenic versions of antibodies, such as chimeric, humanized, and fully human antibodies, in various forms or sophisticated purification of NK cells will be required for *ex vivo* NK cell expansion to overcome the issue of heterophile antibody interference [17, 18]. It is essential to appropriately evaluate cell quality, purity, and safety.

Additionally, novel methods for detecting interference should be evaluated and incorporated in immunoassay systems [8].

In conclusion, our study showed the occurrence of falsely abnormal laboratory test results owing to heterophile antibody interference associated with NK cell therapy for the first time. Information regarding this possibility is particularly important in patients with cancer likely to undergo NK cell therapy, as it may lead to a false diagnosis of recurrence and unnecessary evaluation. Physicians should always consider that NK cell therapy can result in the production of HAMA and be aware of potential problems that may result from this. In addition, safer approaches are needed to demonstrate the benefits of NK cell therapy in future cancer treatment.

Acknowledgments

We would like to thank Editage (www.editage.co.kr) for English language editing.

Disclosure

None of the authors have any potential conflicts of interest associated with research.

References

- Hu W, Wang G, Huang D, Sui M, Xu Y (2019) Cancer immunotherapy based on natural killer cells: current progress and new opportunities. *Front Immunol* 10: 1205.
- Taefehshokr N, Baradaran B, Baghbanzadeh A, Taefehshokr S (2020) Promising approaches in cancer immunotherapy. *Immunobiology* 225: 151875.
- Vivier E, Raulet DH, Moretta A, Caligiuri MA, Zitvogel L, *et al.* (2011) Innate or adaptive immunity? The example of natural killer cells. *Science* 331: 44–49.
- Oh S, Lee JH, Kwack K, Choi SW (2019) Natural killer cell therapy: a new treatment paradigm for solid tumors. *Cancers (Basel)* 11: 1534.
- Liem NT, Van Phong N, Kien NT, Anh BV, Huyen TL, *et al.* (2019) Phase I clinical trial using autologous *ex vivo* expanded NK cells and cytotoxic T lymphocytes for cancer treatment in Vietnam. *Int J Mol Sci* 20: 3166.
- Suen WC, Lee WY, Leung KT, Pan XH, Li G (2018) Natural killer cell-based cancer immunotherapy: a review on 10 years completed clinical trials. *Cancer Invest* 36: 431–457.
- Preissner CM, O’Kane DJ, Singh RJ, Morris JC, Grebe SK (2003) Phantoms in the assay tube: heterophile antibody interferences in serum thyroglobulin assays. *J Clin Endocrinol Metab* 88: 3069–3074.
- Bolstad N, Warren DJ, Nustad K (2013) Heterophilic antibody interference in immunometric assays. *Best Pract Res Clin Endocrinol Metab* 27: 647–661.
- Spencer CA, Lopresti JS (2008) Measuring thyroglobulin and thyroglobulin autoantibody in patients with differentiated thyroid cancer. *Nat Clin Pract Endocrinol Metab* 4: 223–233.
- Rodgers SE, Lew JI (2011) The parathyroid hormone assay. *Endocr Pract* 17 Suppl 1: 2–6.
- Cavalier E, Carlisi A, Chapelle JP, Orfanos P, Uzan M, *et al.* (2009) Human anti-mouse antibodies interferences in Elecsys PTH assay after OKT3 treatment. *Transplantation* 87: 451–452.
- Terunuma H, Deng X, Nishino N, Watanabe K (2013) NK cell-based autologous immune enhancement therapy (AIET) for cancer. *J Stem Cells Regen Med* 9: 9–13.
- Ding L, Shankara-Narayana N, Wood C, Ward P, Sidhu S, *et al.* (2013) Markedly elevated serum thyroglobulin associated with heterophile antibodies: a cautionary tale. *Thyroid* 23: 771–772.
- Serei VD, Marshall I, Carayannopoulos MO (2019) Heterophile antibody interference affecting multiple Roche immunoassays: a case study. *Clin Chim Acta* 497: 125–129.

15. Laudes M, Frohnert J, Ivanova K, Wandinger KP (2019) PTH immunoassay interference due to human anti-mouse antibodies in a subject with obesity with normal parathyroid function. *J Clin Endocrinol Metab* 104: 5840–5842.
16. Hoffman T (1990) Anticipating, recognizing, and preventing hazards associated with *in vivo* use of monoclonal antibodies: special considerations related to human anti-mouse antibodies. *Cancer Res* 50: 1049s–1050s.
17. Akbari B, Farajnia S, Ahdi Khosroshahi S, Safari F, Yousefi M, *et al.* (2017) Immunotoxins in cancer therapy: review and update. *Int Rev Immunol* 36: 207–219.
18. Hong G, Chen X, Sun X, Zhou M, Liu B, *et al.* (2019) Effect of autologous NK cell immunotherapy on advanced lung adenocarcinoma with EGFR mutations. *Precis Clin Med* 2: 235–245.