



HAL
open science

Effect of ceftobiprole on the normal human intestinal microflora

Tobias Bäckström, Georgios Panagiotidis, Olof Beck, Charlotte Asker-Hagelberg, Mamun-Ur Rashid, Andrej Weintraub, Carl Erik Nord

► **To cite this version:**

Tobias Bäckström, Georgios Panagiotidis, Olof Beck, Charlotte Asker-Hagelberg, Mamun-Ur Rashid, et al.. Effect of ceftobiprole on the normal human intestinal microflora. *International Journal of Antimicrobial Agents*, 2010, 36 (6), pp.537. 10.1016/j.ijantimicag.2010.07.021 . hal-00640447

HAL Id: hal-00640447

<https://hal.science/hal-00640447>

Submitted on 12 Nov 2011

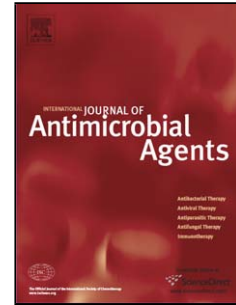
HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Accepted Manuscript

Title: Effect of ceftobiprole on the normal human intestinal microflora

Authors: Tobias Bäckström, Georgios Panagiotidis, Olof Beck, Charlotte Asker-Hagelberg, Mamun-Ur Rashid, Andrej Weintraub, Carl Erik Nord



PII: S0924-8579(10)00353-5
DOI: doi:10.1016/j.ijantimicag.2010.07.021
Reference: ANTAGE 3410

To appear in: *International Journal of Antimicrobial Agents*

Received date: 27-5-2010
Revised date: 27-7-2010
Accepted date: 28-7-2010

Please cite this article as: Bäckström T, Panagiotidis G, Beck O, Asker-Hagelberg C, Rashid M-U, Weintraub A, Nord CE, Effect of ceftobiprole on the normal human intestinal microflora, *International Journal of Antimicrobial Agents* (2010), doi:10.1016/j.ijantimicag.2010.07.021

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Effect of ceftobiprole on the normal human intestinal microflora

Tobias Bäckström, Georgios Panagiotidis, Olof Beck, Charlotte Asker-Hagelberg,
Mamun-Ur Rashid, Andrej Weintraub, Carl Erik Nord *

*Department of Laboratory Medicine, Karolinska University Hospital, Karolinska
Institutet, SE-141 86 Stockholm, Sweden*

ARTICLE INFO

Article history:

Received 27 May 2010

Accepted 28 July 2010

Keywords:

Ceftobiprole

Normal human intestinal microflora

* Corresponding author. Tel.: +46 8 585 878 38; fax: +46 8 585 879 33.

E-mail address: carl.erik.nord@ki.se (C.E. Nord).

ABSTRACT

Ceftobiprole is a new broad-spectrum pyrrolidinone cephem active against meticillin-resistant *Staphylococcus aureus*, vancomycin-resistant *Enterococcus faecalis* and Gram-negative bacteria such as Enterobacteriaceae and *Pseudomonas* spp. The purpose of the present study was to investigate the effect of administration of ceftobiprole on the normal intestinal microflora. Twelve healthy subjects (six males and six females) aged 20–31 years received ceftobiprole 500 mg by intravenous infusion every 8 h for 7 days. Plasma samples were collected on Days –1, 1, 4, 7, 10, 14 and 21 for determination of drug concentration by biological and chemical methods. Faecal samples were collected on Days –1, 2, 4, 7, 10, 14 and 21. For analysis of the microflora, faecal specimens were cultured on non-selective and selective media. Different colony types were counted, isolated in pure culture and identified to genus level. All new colonising aerobic and anaerobic bacteria were tested for susceptibility to ceftobiprole. Plasma concentrations of ceftobiprole 10 min after completion of infusion were as follows: Day 1, 14.7–23.6 mg/L; Day 4, 15.9–24.5 mg/L; and Day 7, 15.9–23.9 mg/L. No ceftobiprole was detected in plasma on Days –1, 10, 14 and 21. No measurable concentrations of ceftobiprole were found in faeces on Days –1, 2, 4, 7, 10, 14 and 21. There were minor changes in the numbers of enteric bacteria, enterococci and *Candida albicans* and there were moderate changes in the numbers of bifidobacteria, lactobacilli, clostridia and *Bacteroides* spp. during the same period. No *Clostridium difficile* strains or toxins were found. No new colonising aerobic and anaerobic bacteria with ceftobiprole minimum inhibitory concentrations of ≥ 4 mg/L were found. Ceftobiprole had no significant ecological impact on the human intestinal microflora.

1. Introduction

Under usual circumstances, the normal human microflora is relatively stable in each ecological habitat and acts as a barrier against colonisation by potentially pathogenic microorganisms. Disturbances in the normal microflora may occur due to changes in diet, radiation or administration of antimicrobial agents. Administration of antimicrobial agents can cause several adverse effects on the microflora. Emergence of resistance among bacteria in the normal flora and distribution of resistance genes by transfer of DNA in the microbial community can contribute to an increased load of resistant, potentially pathogenic microorganisms. Another effect of a disturbed normal microflora is reduction in colonisation leading to overgrowth of already present microorganisms or of exogenic pathogens [1]. The extent of disturbances is influenced by the spectrum of the agent, dose, route of administration, pharmacokinetic and pharmacodynamic properties and in vivo inactivation of the agent. Parenterally administered agents that are excreted in the bile or the intestinal mucosa may interfere with the normal intestinal microflora [2].

Increased resistance in many Gram-positive bacteria has driven the need to develop new antibacterial agents with activity against these pathogens, including methicillin-resistant *Staphylococcus aureus* (MRSA). Ceftobiprole is a new broad-spectrum pyrrolidinone cephem active against Gram-positive bacteria including *S. aureus* (MRSA and methicillin-susceptible), vancomycin-resistant *Enterococcus faecalis* and Gram-negative bacteria such as Enterobacteriaceae and *Pseudomonas* spp. Ceftobiprole has undergone extensive evaluation in phase I studies to characterise dose, pharmacokinetics and safety/tolerability as well as in an early phase II study and subsequent phase III studies. It is the first cephalosporin to demonstrate clinical

efficacy in patients with infections due to MRSA and is expected to be a useful addition to the armamentarium of agents for the treatment of complicated skin infections and pneumonia [3].

The purpose of the present study was to investigate the effect of ceftobiprole on the intestinal microflora over 7 days of administration. Careful investigation of the effect of antibiotic treatment on the endogenous microflora is of importance since alteration of the endogenous flora balance, qualitatively and/or quantitatively, may facilitate colonisation by new potentially pathogenic strains or enable microorganisms already present in the normal flora to develop resistance. Pharmacokinetics and pharmacodynamics are further important consideration for the safety and efficacy of antimicrobial agents [4].

2. Materials and methods

2.1. Objectives

The primary objective was to assess the effect of antibiotic treatment on the intestinal microflora before, during and after administration of ceftobiprole given to healthy individuals.

Secondary objectives were: (i) to explore the potential for development of resistance by measuring the susceptibility [minimum inhibitory concentrations (MICs)] of new colonising isolated bacterial strains before, during and after antibiotic treatment; (ii) to correlate intestinal microflora patterns with ceftobiprole concentrations measured in

plasma and faeces; and (iii) to investigate the concentration of ceftobiprole in faeces compared with plasma.

2.2. Study design

This was an open-label, non-comparative, multiple-dose, single-centre study in 12 healthy subjects. Each subject received 500 mg of ceftobiprole by intravenous (i.v.) infusion over 120 min every 8 h (q8h) for 7 days. Subjects were admitted to the study centre the day before the first drug administration and were discharged from the study centre on Day 8. Subjects visited the study centre for follow-up visits on Days 10, 14 and 21. A total of 13 plasma samples were collected from each subject, including 1 at pre dose (Day -1), 3 samples each on Days 1, 4 and 7 and 1 sample each on Days 10, 14 and 21. A total of seven faecal samples were collected, including at pre-dose (Day -1) and on Days 2, 4, 7, 10, 14 and 21. Each subject included in the study participated at follow-up visits on Days 10, 14 and 21. Additional safety evaluations included physical examination, vital signs, clinical laboratory tests, 12-lead electrocardiogram (ECG) and adverse event monitoring.

2.3. Subjects

Twelve healthy subjects (six males and six females) aged 20–31 years were included in the study. They were recruited through information about the study on the Clinical Pharmacology Trial Unit website on the internet. Physical examination was carried out on each subject before the start of the study, including measurements of blood pressure and heart rate, ECG and clinical laboratory safety tests as well as an interview on medical and surgical history. Female subjects were tested for

pregnancy. Inclusion criteria were men and women aged between 18 years and 45 years and normal findings in the medical history and physical examination. Exclusion criteria were: regular use of medication except contraceptive tablets; treatment with antimicrobial agents within the 3 months preceding the study; participation in a trial with another investigational drug within 1 month preceding the study; present or residual gastrointestinal, liver or kidney disease, or other conditions known to interfere with the absorption, distribution, metabolism or excretion of drugs; known history of *Clostridium difficile* infection; history of hypersensitivity to β -lactam antibiotics; pregnant or breast-feeding women; estimated creatinine clearance of <80 mL/min; positive screen for hepatitis B or C or human immunodeficiency virus (HIV); history of alcohol abuse or drug abuse; and donation of blood or blood products within 1 month preceding the study. Before being admitted to the study, subjects gave consent to participate after the nature, scope and possible consequences of the study had been explained in a form understandable to him or her. The subject was also given written information and a copy of the signed consent form.

2.4. Approvals

The study protocol submitted to the Ethics Committee of Karolinska Institutet (Stockholm, Sweden) and to the Medical Products Agency (Uppsala, Sweden) was approved before starting the trial.

2.5. Drug administration

Subjects each received ceftobiprole 500 mg by i.v. infusion over 120 min q8h for 7 days.

2.6. Sampling of specimens

Plasma samples were obtained for chemical analysis of ceftobiprole on study Day – 1, after the start of the first ceftobiprole infusion on study Days 1, 4 and 7 and on study Days 10, 14 and 21.

Samples were obtained for bioassay and chemical analysis of ceftobiprole from faecal specimens passed on study Days –1, 2, 4, 7, 10, 14 and 21, unless no specimen was passed on a given day, in which instance the first subsequent specimen passed after that day, before the next collection time point, was collected. If more than one specimen was produced on a specified day, only the first specimen on that day was collected.

Plasma and faecal samples for the analyses were collected in sterile tubes and containers and were frozen at –70 °C until processed.

2.7. Determination of ceftobiprole plasma and faecal concentrations

Plasma and faecal concentrations of ceftobiprole were determined by the following developed and validated methods by liquid chromatography–tandem mass spectrometry. Concentrations of ceftobiprole in Li-heparin human plasma (pH 5) were determined using an ultra performance liquid chromatography–tandem mass spectrometry (UPLC-MS/MS) system (Waters Corp., Milford, MA) following sample preparation by protein precipitation with 1% formic acid in acetonitrile containing BAL0009141-d4 as an internal standard. The final extract of plasma sample was

injected into the UPLC-MS/MS system. Concentrations of ceftobiprole in faecal extract (supernatant from human faecal acidic homogenised with phosphate-buffered saline solution) were determined following sample preparation by precipitation with 1% formic acid in acetonitrile containing BAL0009141-d4 as an internal standard. The supernatant was diluted with the same volume of 1% formic acid and injected into the UPLC-MS/MS system. Separation of the analytes was achieved on an ACQUITY UPLC BEH C₁₈ column (1.7 µm, 2.1 × 50 mm) (Waters Corp.) using a gradient run with mobile phase A of 0.1% formic acid in water and mobile phase B of acetonitrile. Analytes were detected using a Micromass Quattro Premier XE mass spectrometer (Waters Corp.) operating in positive electrospray ionisation mode utilising multiple reaction monitoring for the transitions 535→203 m/z for ceftobiprole and 539→207 m/z for IS (International System of Units). The calibration curve in plasma was linear over the range 0.05–30 mg/L and in faecal extract over the range 1.00–143 mg/kg faeces. Ceftobiprole concentrations in faeces were also biologically determined in Antibiotic Medium 1 (Difco, Sparks, MD) with *Micrococcus luteus* ATCC 9341 as indicator strain [5]. Two validation reports were provided by the investigator giving information on linearity ranges, lower limit of detection as well as intra-assay and inter-assay coefficient of variation. Standards were prepared in faeces for faecal samples.

2.8. Processing of faecal specimens for microbiological analyses

Samples were obtained for culture and ceftobiprole susceptibility tests from faecal specimens passed on study Days –1, 2, 4, 7, 10, 14 and 21, unless no specimen was passed on a given day in which instance the first subsequent specimen passed was collected. Samples were suspended in pre-reduced peptone yeast extract medium,

diluted ten-fold and inoculated on non-selective and selective agars as described by Nord et al. [6]. Aerobic agar plates were incubated for 24 h at 37 °C and anaerobic plates were incubated for 48 h at 37 °C in anaerobic jars (BBL GasPak™; BD, Cockeysville, MD). Following incubation, different colony types were counted, isolated in pure culture and identified to genus level. All isolates were analysed according to Gram-stain appearance and colony morphology followed by biochemical tests [7]. Anaerobic microorganisms were identified by gas chromatographic analysis of metabolites from glucose. *Clostridium difficile* strains were further characterised by cytotoxicity assay, polymerase chain reaction (PCR) ribotyping and a multiplex real-time PCR (Xpert™ *C. difficile*; Cepheid, San Francisco, CA) as described recently [8]. The lower limit of detection for faeces was 10² colony-forming units (CFU)/g.

2.9. Ceftobiprole susceptibility tests

MICs for ceftobiprole were determined for new colonising strains from the faecal samples by the agar dilution method according to Clinical and Laboratory Standards Institute (CLSI) guidelines [9,10]. The final inoculum was 10⁴ CFU/spot for aerobic bacteria and 10⁵ CFU/spot for anaerobic bacteria. Inoculated plates were incubated for 24 h (aerobic bacteria) and 48 h (anaerobic bacteria). Reference strains were *S. aureus* ATCC 29213, *E. faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Bacteroides fragilis* ATCC 25285 and *C. difficile* ATCC 700057. Isolated strains were considered resistant according to CLSI breakpoints (recommended breakpoint for ceftobiprole is 4 mg/L). The MIC was defined as the lowest concentration of drug that inhibited growth completely. MIC₅₀ and MIC₉₀ values corresponded to the concentrations that inhibited the growth of 50% and 90% of the strains tested, respectively.

2.10. Safety

The following safety assessments were performed: physical examination on study Days –1, 1, 4, 7 and 10 as well as follow-up visit (Day 14) and end-of-study visit (Day 21); vital signs; ECG (Days –1 and 21); adverse events monitoring; and monitoring of clinical laboratory parameters.

2.11. Statistical methods

Statistics were calculated for the values estimated for the faecal specimens as log number of microorganisms per gram faeces and faecal concentrations of ceftobiprole as well as for the pharmacokinetic analysis by use of Wilcoxon signed-rank test.

3. Results

3.1. Ceftobiprole concentrations in plasma and faeces

Plasma ceftobiprole concentrations are shown in Table 1. Concentrations in samples taken 10 min after completion of infusion were as follows: Day 1, 14.7–23.6 mg/L (mean 19.4 mg/L); Day 4, 15.9–24.5 mg/L (mean 20.5 mg/L); and Day 7, 15.9–23.9 mg/L (mean 20.3 mg/L). No ceftobiprole was detected in plasma on Days –1, 10, 14 and 21. No measurable faecal concentrations were found on Days –1, 2, 4, 7, 10, 14 and 21.

3.2. Effect of ceftobiprole on the aerobic intestinal microflora

The effect of ceftobiprole on the aerobic intestinal microflora is shown in Fig. 1. Mean counts of *E. coli* decreased by ca. 1.5 log CFU/g of faeces from study Day –1 to study Day 7, with recovery to baseline counts on Day 21. Mean values for Enterobacteriaceae did not change from study Day –1 to study Day 21. Mean numbers of enterococci decreased 1.0 log CFU/g of faeces from Day –1 to Day 7 and then increased 2 log CFU/g of faeces to Day 14; on Day 21 the numbers of enterococci were recovered to baseline. The numbers of *Candida albicans* were within the normal variation. Changes in the aerobic intestinal microflora (≤ 2 log CFU/g of faeces) were not significant.

3.3. Effect of ceftobiprole on the anaerobic intestinal microflora

The effect of ceftobiprole on the anaerobic intestinal microflora is shown in Fig. 2. There were no changes in the numbers of lactobacilli and bifidobacteria from Day –1 to Day 21. Counts of clostridia increased from Day 2 to Day 7 by ca. 1.5 log CFU/g of faeces and then returned to baseline counts. The numbers of *Bacteroides* were only influenced on Day 2, with a decrease of ca. 0.5 log CFU/g of faeces. All alterations were within the normal variation and significance. No *C. difficile* strains or toxins A and B were found.

3.4. Ceftobiprole susceptibility tests

No new colonising aerobic and anaerobic bacteria resistant to ceftobiprole (MIC ≥ 4 mg/L) were found.

3.5. Safety and tolerability

There were 10 subjects with 28 adverse events, all mild in severity. Four events of common cold, one event of very mild swelling of the lower lip, one event of vasovagal reaction in relation to i.v. cannula insertion and one event of muscle pain were deemed to be unrelated to the study drug. Other adverse events were deemed to be possibly related to ceftobiprole and included infusion-site reactions in three subjects, with pain, mild swelling and thrombophlebitis in one subject. Mild rash was seen in three subjects and vaginal *Candida* infection in two subjects. Two subjects had two events of headache each. One subject had two events of mild diarrhoea. Nausea was seen in one subject. During study drug infusion, five subjects noticed mild taste alterations. No subjects had potentially clinically significant changes in post-baseline vital sign values. No subjects had potentially clinically significant changes in post-baseline haematology, chemistry or urine analysis based both on normal ranges and on percent change from baseline. There were no significant changes in ECG parameters from baseline to post baseline in any subject.

4. Discussion

Ceftobiprole is active against most aerobic Gram-positive bacteria as well as against many Enterobacteriaceae and *Pseudomonas aeruginosa* strains. Anaerobic Gram-positive bacteria such as bifidobacteria, propionibacteria and peptostreptococci are susceptible, whilst clostridia are variable in susceptibility to ceftobiprole. The MIC for *C. difficile* strains is between 1.0 mg/L and 8.0 mg/L. *Bacteroides fragilis* and *Prevotella* spp. are resistant to ceftobiprole [11].

Ceftobiprole is a promising antimicrobial agent for treatment of complicated skin infections and pneumonia. Ceftobiprole is reported to be well tolerated with good safety, which was also observed in the present investigation [12].

Administration of antibacterial agents can cause disturbances in the ecological balance between the host and microorganisms. These changes are dependent on the spectrum of activity, dose, route of administration, pharmacokinetic and pharmacodynamic properties and in vitro inactivation of the agent. Secretion of an agent by intestinal mucosa or bile may have an impact on the intestinal microflora leading to antibiotic resistance. The effect of ceftobiprole on the normal intestinal microflora has not been studied previously. No faecal concentration of ceftobiprole was found and the minor effect on the intestinal microflora is thus explained by these findings. Ceftobiprole is mainly eliminated by renal excretion [13]. No new colonising ceftobiprole-resistant aerobic and anaerobic bacteria in the normal intestinal flora were recovered, probably owing to less selective pressure for the emergence of colonisation by resistant microorganisms. *Clostridium difficile* infection (CDI) is an adverse event mainly associated with antibiotic treatment and prophylaxis. Broad-spectrum antibiotics such as cephalosporins, fluoroquinolones and amoxicillin are most often involved in causing this infection. In the present trial, no clinical *C. difficile* infection was observed, probably due to no biological activity of ceftobiprole in the intestine. Based on these findings, ceftobiprole has a favourable ecological profile. However, when ceftobiprole is used in hospitalised patients with serious infections and pre-existing *C. difficile* strains, the risk of development of CDI should be monitored.

Funding

This study was funded by Janssen-Cilag Medical Affairs EMEA, a division of Janssen Pharmaceutica N.V.

Competing interests

None declared.

Ethical approval

The study protocol was approved by the Ethics Committee of Karolinska Institute (Stockholm, Sweden) and the Medical Products Agency (Uppsala, Sweden).

Accepted Manuscript

References

- [1] Sullivan Å, Edlund C, Nord CE. Interaction between antimicrobial agents and the oropharyngeal and intestinal microflora. In: Bryskier A, editor. *Antimicrobial agents*. Washington, DC: ASM Press; 2005. p. 1357–70.
- [2] Sullivan Å, Edlund C, Nord CE. Effect of antimicrobial agents on the ecological balance of human microflora. *Lancet Infect Dis* 2001;1:101–14.
- [3] Barbour A, Schmidt S, Rand KH, Derendorf H. Ceftobiprole: a novel cephalosporin with activity against Gram-positive and Gram-negative pathogens, including methicillin-resistant *Staphylococcus aureus* (MRSA). *Int J Antimicrob Agents* 2009;34:1–7.
- [4] The European Agency for the Evaluation of Medicinal Products, Committee for Proprietary Medicinal Products (CPMP). Points to consider on pharmacokinetics and pharmacodynamics in the development of antibacterial medicinal products. London, UK: EMEA; 2000. CPMP/EWP/2655/99.
- [5] Panagiotidis G, Bäckström T, Asker-Hagelberg C, Jandourek A, Weintraub A, Nord CE. Effect of ceftaroline on normal human intestinal microflora. *Antimicrob Agents Chemother* 2010;54:1811–4.
- [6] Nord CE, Rasmanis G, Wahlund E. Effect of dalbavancin on the normal intestinal microflora. *J Antimicrob Chemother* 2006;58:627–31.
- [7] Murray PR, Baron EJ, Jorgensen JH, Landry ML, Pfaller MA, editors. *Manual of clinical microbiology*. 9th ed. Washington, DC: ASM Press; 2007.
- [8] Huang H, Weintraub A, Fang H, Nord CE. Comparison of a commercial multiplex real-time PCR to the cell cytotoxicity neutralization assay for diagnosis of *Clostridium difficile* infections. *J Clin Microbiol* 2009;47:3729–31.

- [9] Clinical and Laboratory Standards Institute. *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard*. 7th ed. Document M7-A7. Wayne, PA: CLSI; 2006.
- [10] Clinical and Laboratory Standards Institute. *Methods for antimicrobial susceptibility testing of anaerobic bacteria; approved standard*. 7th ed. Document M11-A7. Wayne, PA: CLSI; 2007.
- [11] Ednie L, Shapiro S, Appelbaum PC. Antianaerobe activity of ceftobiprole: a new broad-spectrum cephalosporin. *Diagn Microbiol Infect Dis* 2007;58:133–6.
- [12] Schirmer PL, Deresinski SG. Ceftobiprole: a new cephalosporin for the treatment of skin and skin structure infections. *Expert Rev Anti Infect Ther* 2009;7:777–91.
- [13] Murthy B, Schmitt-Hoffmann A. Pharmacokinetics and pharmacodynamics of ceftobiprole, an anti-MRSA cephalosporin with broad-spectrum activity. *Clin Pharmacokinet* 2008;47:21–33.

Fig. 1. Effect of ceftobiprole administration on the aerobic intestinal microflora of 12 healthy subjects. Solid line represents mean value of logarithmic number of microorganisms/g of faeces.

Fig. 2. Effect of ceftobiprole administration on the anaerobic intestinal microflora of 12 healthy subjects. Solid line represents mean value of logarithmic number of bacteria/g of faeces.

Accepted Manuscript

Table 1

Ceftobiprole plasma concentrations 10 min after completion of infusion in 12 subjects receiving 500 mg doses of ceftobiprole intravenously every 8 h for 7 days

Subject	Ceftobiprole concentration (mg/L)						
	Day -1	Day 1	Day 4	Day 7	Day 10	Day 14	Day 21
1	N/D	19.5	22.0	18.0	N/D	N/D	N/D
2	N/D	14.7	17.3	15.9	N/D	N/D	N/D
3	N/D	20.4	23.1	20.7	N/D	N/D	N/D
4	N/D	15.6	21.8	20.8	N/D	N/D	N/D
5	N/D	22.9	23.1	21.9	N/D	N/D	N/D
6	N/D	15.3	16.9	20.1	N/D	N/D	N/D
7	N/D	20.9	18.7	19.9	N/D	N/D	N/D
8	N/D	22.5	21.3	21.7	N/D	N/D	N/D
9	N/D	21.4	22.7	21.9	N/D	N/D	N/D
10	N/D	18.1	15.9	18.6	N/D	N/D	N/D
11	N/D	23.6	24.5	23.9	N/D	N/D	N/D
12	N/D	18.1	18.5	19.7	N/D	N/D	N/D
Range	–	14.7–23.6	15.9–24.5	15.9–23.9	–	–	–
Mean	–	19.4	20.5	20.3	–	–	–

N/D, not detected.

