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Vancomycin-associated nephrotoxicity, a case control study

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Contents

Abstract

Background: Vancomycin is a cornerstone antibiotic for the management of severe Gram positive infections. However, high doses of vancomycin are associated with a risk of nephrotoxicity. This study aimed to evaluate the relationship between the evolution of vancomycin trough concentration and the occurrence of nephrotoxicity, and to identify risk factors for both vancomycin-associated nephrotoxicity and vancomycin overexposure.

Methods: A total of 1240 patients records from our hospital therapeutic drug monitoring database between 2007 and 2011 were screened and grouped according to predefined criteria defining vancomycin overexposure (one or more occurrence of a trough level ≥ 20 mg/L) and treatment-related nephrotoxicity (rise of serum creatinine by ≥ 50% over baseline). A representative sample of 150 cases was selected for in depth analysis. Weighted logistic regression analyses were used to test associations between vancomycin overexposure, nephrotoxicity and other predictors of interest.

Results: Patients with high trough concentrations were found to be more likely to develop nephrotoxicity (odds ratio: 4.12; $p < 0.001$). Specific risk factors, notably concomitant nephrotoxic treatments and comorbid conditions (heart failure), were found to independently increase the risk of either nephrotoxicity or vancomycin exposure. Finally, the exploration of temporal relationships between variations of vancomycin trough concentrations and creatinine levels were in line with circular causality with some antecedence of vancomycin on creatinine changes.

Conclusion: Our results confirm the important nephrotoxic potential of vancomycin and indicate that the utilisation of this drug deserves thorough individualization for conditions susceptible to increase its concentration exposure and reactive adjustment based on therapeutic drug monitoring.

Background

1. Vancomycin: a half century of fighting infection.

Vancomycin is a tricyclic glycopeptide antibiotic discovered in 1953. It is a large and complex molecule with a molecular weight of approximately 1500 Daltons, naturally produced by an Actinobacterium species called *Streptomyces orientalis.* It has been licensed for clinical use since 1958. However, adverse effects due to impurities in early formulations of vancomycin, humorously called "Mississippi mud", restrained its expansion in the clinical world (1,2). Renewed interest for vancomycin appeared with the emergence of resistance to beta-lactam antibiotics. Beta-lactam resistant bacteria, such as Methicillin-resistant *Staphylococcus aureus* (MRSA), are important causative agents of both community-acquired and nosocomial infections today. Treatment options for these infections are limited and less effective than options available for non-resistant bacteria, resulting in higher morbidity and mortality (3,4,5). Vancomycin is nowadays a keystone for the control of severe Gram-positive infections, including those due to beta-lactam resistant pathogens (2,6,7,8).

This effectiveness is explained by a different mechanism of action compared to classic beta-lactam antibiotics, although both types of drugs inhibit cell wall synthesis. Beta-lactam antibiotics bind to transpeptidase enzymes, which catalyse the growth of the peptidoglycan layer, a major component of cell walls. Vancomycin binds directly to a cell wall precursor, preventing its incorporation into the peptidoglycan layer (8,9). The bactericidal effect resulting from this inhibition of cell wall synthesis is quite slow to develop. Vancomycin is so known as a "time-dependent killer", meaning that it is effective only if the antibiotic's concentration at the target site remains higher than the minimum inhibitory concentration (MIC) of targeted pathogen for the longest possible time (9,10). Vancomycin effectiveness has also been correlated with the ratio of the area under serum vancomycin concentration curve (AUC) over the MIC. An AUC/MIC ratio >400 is associated with better outcome in complicated infections (7,11).

Considering pharmacokinetic parameters, vancomycin is poorly absorbed from the gastrointestinal tract. The main route of administration is therefore the intravenous route, except for the treatment of *Clostridium difficile* colitis where oral therapy is indicated to produce local bacterial killing in the gut lumen. The binding of vancomycin to plasma proteins is around 55% and its volume of distribution ranges from 0.4 to 1 L/kg. Mean elimination half-life from plasma is approximately 4 to 6 hours in patients with normal renal function. Vancomycin is not metabolized in the body and mainly eliminated by the kidneys via glomerular filtration. Biliary excretion is a minor (5-10%) excretion pathway of vancomycin (8,9,12).

Vancomycin has a wide spectrum of action on Gram-positive bacteria, being effective on most of *Staphylococcus, Streptococcus, Enterococcus* and *Clostridium* species. Due its molecular size, it cannot cross the outer layer of Gram-negative bacteria, thus lacking efficacy against them (1,8). Vancomycin is usually administrated by intermittent intravenous infusions every 6 to 12 hours. Continuous infusion is another administration option. Despite faster attainment of therapeutic concentrations using continuous infusion, no significant advantages were shown regarding clinical outcomes. (1,13) Administration should be slow (infusion rate <1g/h) to avoid pseudo-hypersensitivity reactions such as the "red man syndrome".

As it happened with other antibiotics, resistance is also emerging against vancomycin. Some occurrences of *Staphylococcus aureus* strains, including MRSA, revealing resistance to vancomycin have been reported several times in the United States since the beginning of the 21st century. Resistance has also been described involving Enterococcus species or other Staphylococcus species, such as the VRSE (Vancomycin resistant *Staphylococcus epidermidis*). This resistance phenomenon is quite a matter of concern, as recent guidelines for the treatment of severe Gram-positive infections, especially those due to MRSA, enacted by the Infectious Diseases Society of America (IDSA) widely recommend vancomycin use (8,9,14,15).

In a 2009 major consensus review (9), the American Society of Health-System Pharmacists, the Infectious Diseases society of America and the Society of Infectious diseases Pharmacists have established guidelines for vancomycin therapy and therapeutic drug monitoring (TDM). Trough concentrations (i.e. residual levels of vancomycin in plasma at the end of a dosage interval) should always be maintained >10 mg/L to avoid the development of resistance (16). The traditional dosage for an adult without renal dysfunction is 1 g every 12 hours (15 mg/kg b.i.d.) (9). The target trough concentration proposed in complicated infections, such as MRSA infections, is 15 – 20 mg/L. Higher doses than the classical 1 g b.i.d. often need to be given to reach this level of exposure. According to the consensus guideline, TDM of vancomycin should be performed using trough concentrations rather than peak concentrations and the monitoring samples should be drawn just before the fourth dose administration under steady regimen, at least for patients with normal renal function. Monitoring should be performed on a weekly basis, although severe clinical conditions and known, or suspected, renal dysfunction indicate for more frequent monitoring. (9,17-20)

As previously said, vancomycin is known since its first use in clinics for adverse effects. As it is very irritating for tissues, local complications such as or thrombophlebitis or even necrosis (in case of extravascular administration) can occur. Hypersensitivity-like reactions are also described, such as the "red-man syndrome" in which patient develops flush or maculopapular rash localized most frequently on face, neck, chest and upper limb, due to histamine release from granulocytes. In rare cases, a severe

reaction can occur with blood pressure collapse, dyspnea or angioedema, becoming life-threatening for patient. Rare adverse effects involving gastrointestinal tract (pseudomembranous colitis) and haematological disorders (leucopoenia, thrombocytopenia) are also described (8). But the most frequent concerns regarding vancomycin therapy remain ototoxicity and nephrotoxicity. Cochlear ototoxicity is indeed reported to occur with 2-5% of patients. It results from dose and duration-dependent toxicity, thus its occurrence is usually associated with high plasmatic concentrations of vancomycin (greater than 60 mg/L) over prolonged times (8,9).

2. Nephrotoxicity: the other side of the coin.

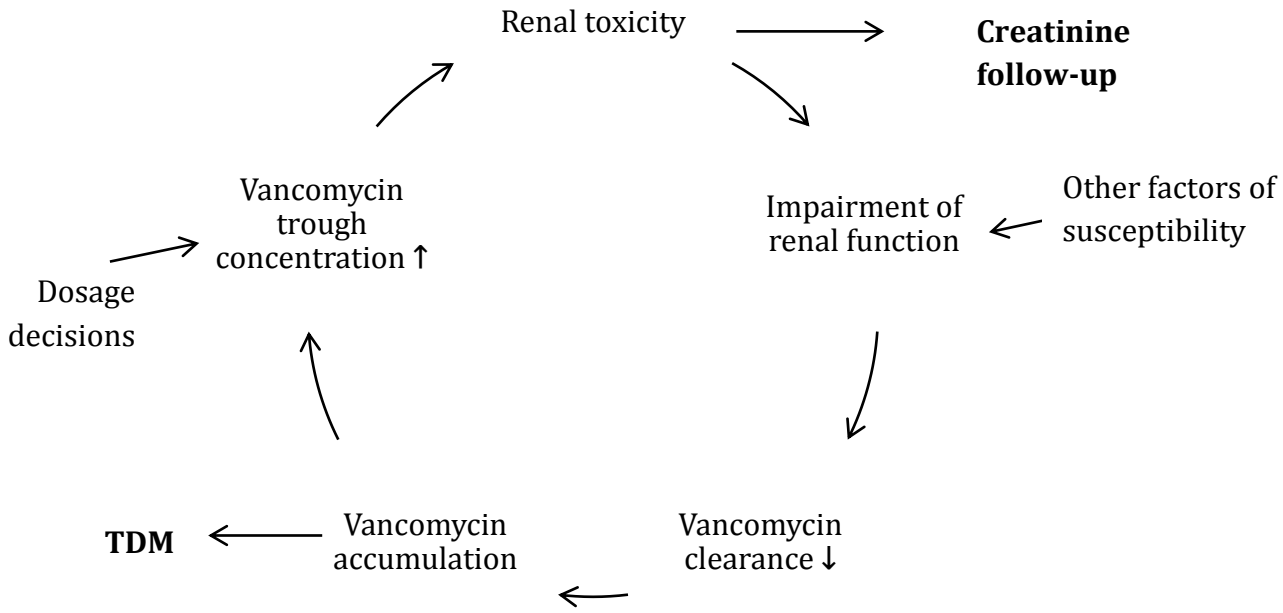
Vancomycin-associated nephrotoxicity remains an important matter of concern since vancomycin has been approved for clinical use. As said before, impurity in early formulations was associated with a high occurrence of nephrotoxicity. Nowadays, impurities are not a problem anymore, but nephrotoxicity is still known as a frequent adverse effect of vancomycin therapy. As of today, this is well documented and described through many studies and reports (1-2,6-9,11,21).

In both studies and clinical practice, nephrotoxicity is usually defined as a rise of 50% or more for at least two consecutives serum creatinine measurements over baseline, that occurs during vancomycin therapy or during 72h after vancomycin interruption (1,6,7,22). Nephrotoxicity is described as reversible in most of the cases following treatment interruption or adjustment. (7-9). Permanent renal damage is infrequently reported. Acute tubular necrosis is the main toxic action observed in kidney. The mechanism leading to nephrotoxicity remains unclear but seems to be related with oxidative stress on renal proximal tubule cells after intracellular penetration of vancomycin. Some studies have indeed shown increased oxygen consumption in those cells after vancomycin administration. Cases of interstitial nephritis involving an immuno-allergic mechanism have also been reported (2,7,21,23,25).

Emergence of resistant pathogens, such as *Methicillin-Resistant Staphylococcus Aureus* (MRSA), has led to target higher vancomycin concentrations for ensuring therapeutic effect. However, it is also known that the administration of doses required to achieve the higher exposure targeted in this condition increases significantly the risk of nephrotoxicity (2,6,7,11,22). Incidence of vancomycin-associated nephrotoxicity is quite variable between studies but it is most commonly reported to occur in 10-20% of patients with a targeted trough-concentration 10-15 mg/L, but in 30-40% of patients with high doses therapy (targeted trough 15-20 mg/L) (1,2,5,16,21). Available observations suggest an increased risk of nephrotoxicity for vancomycin trough-concentrations over 20 mg/L or daily doses >4g (7,24). Concomitant exposure to other known nephrotoxic agents or to treatments causing pre-renal dysfunction, such as aminoglycosides antibiotics or diuretics, is also described as a risk factor for induced nephrotoxicity. (1,2,5,6,11,14,26)

As vancomycin is both eliminated by and toxic to the kidneys, there is thus a bidirectional relationship between nephrotoxicity and vancomycin overexposure. Indeed, during the recent past, hospital consultants from the Division of Clinical Pharmacology have been referred a certain number of patients for the development of renal failure occurring under vancomycin treatment and associated with high trough concentrations. Clinically, the causation link between overexposure and nephrotoxicity is quite difficult to assess, as they both are the consequence from one another but could both contribute to the occurrence of the other. This could be described as a vicious circle, bringing out a "chicken-and-egg" problem: what occurs first?

Fig.1: Vicious circle and ways to interfere with it.



In this context, devising preventive measures to improve the therapeutic use of vancomycin and to control its toxic potential represents a challenging task. A better understanding of how this circular causality works and of how it is further influenced by various risk factors could possibly contribute to improve the recommendations for an effective and safe use of this important antibiotic.

3. Study aims

This retrospective case-control study aims to evaluate the relationship between vancomycin trough concentrations and the development of nephrotoxicity, to identify risk factors associated with vancomycin-associated nephrotoxicity, to identify risk factors for vancomycin overexposure itself and to explore the temporal link between the rise of vancomycin trough concentrations and the rise of serum

creatinine levels. Recommendations regarding monitoring and adjustments during vancomycin therapy could eventually be brought out from these observations.

Methods

1. Study design and data collection

This retrospective case-control study was conducted in the University Hospital of Lausanne (CHUV) in Switzerland. The Ethics Committee of the Faculty of Biology and Medicine in Lausanne has approved the study. Patients were recruited from the hospital laboratory database (MOLIS, Vision4Health, CompuGroup Medical, Oechsli 7, 8807 Freienbach, Switzerland). We first identified all patients who received vancomycin therapy between January 2007 and December 2011, and who had at least one vancomycin concentration measurement (recruitment criterion). We included in the study adults patients (≥16 years old), hospitalized in non-intensive care units, and for whom serum creatinine measurements were available in a timespan of one month surrounding first and last recorded vancomycin trough-concentration (selection criteria). A stay in the intensive care unit for less than 72 hours during vancomycin therapy was admitted and did not exclude the patient from the study. Patients who underwent dialysis or for whom blood sampling or vancomycin measurements times were unclear or unknown were excluded.

For patients meeting the selection criteria, we extracted the following data from the laboratory database:

- Date and time of blood sampling for all recorded vancomycin trough-concentrations measurements, with date and time of the last vancomycin administration and vancomycin dosage at the time of blood sampling.
- Date and time of blood sampling for all recorded serum creatinine measurements.
- If recorded, date and time of blood sampling for vancomycin peak-concentrations measurements, with date and time of the last vancomycin administration and vancomycin dosage at the time of blood sampling.

In order to later discriminate between cases and controls, patients were grouped on the basis of two criteria: occurrence of vancomycin overexposure (**V** criterion) and occurrence of vancomycin-associated nephrotoxicity (**C** criterion). Overexposure was defined in our study as the existence of at least one vancomycin trough concentration ≥20 mg/L (**V1; V0** otherwise). Vancomycin-associated nephrotoxicity was defined as a rise by ≥50% of serum creatinine levels from baseline for two consecutive measures, occurring during vancomycin therapy or within 72 hours after therapy interruption (**C1; C0** otherwise).

The identification of patients actually matching **C1** criterion required a two-steps process, as data from MOLIS (laboratory database) did not allow the determination of the period of vancomycin therapy and creatinine baseline. In the first step, we screened the patient with a rise ≥ 50% of creatinine levels between any of two measures in a one-month timespan surrounding first and last recorded vancomycin trough-concentration (**C1'; C0** otherwise). 4 groups of patients were then defined within the "*screened population*":

- Group **V0C0**: vancomycin trough-concentrations <20 mg/L and rise of creatinine by <50% between two measures.
- Group **V0C1'**: vancomycin trough-concentrations <20 mg/L and rise of creatinine by ≥50% between two measures.
- Group **V1C0**: at least one vancomycin trough-concentration ≥20 mg/L and rise of creatinine by <50% between two measures.
- Group **V1C1':** at least one vancomycin trough-concentration ≥20 mg/L and rise of creatinine by ≥50% between two measures.

The second step consisted in a random selection of patients matching **C1'** (i.e patients belonging either to **V0C1'** or **V1C1'** group) for intensive data extraction from the hospital medical records (ARCHIMED database). This closer analysis of patients records was done in order to determine the date of onset and duration of vancomycin therapy, creatinine baseline value and occurrence of vancomycin-associated nephrotoxicity as defined earlier (**C1**). Creatinine baseline value was calculated as the average of the last 3 values of creatinine prior the antibiotic therapy start. Patients who matched the preliminary rough **C1'** criterion but failed to match the final **C1** criterion were excluded. As this two steps process (screening and detailing) was time consuming, we proceeded to review only a fraction of patients in both the **V0C1'** and **V1C1'** groups. In each group, the total number of patients matching the **C1** criterion was extrapolated from the number of records that had to be reviewed to obtain 25 patients matching the **C1** criterion. At the end, the "*reviewed population*" contained 4 groups as follows:

- Group **V0C0**: neither vancomycin overexposure nor vancomycin-associated nephrotoxicity.
- Group **V0C1**: no vancomycin overexposure but vancomycin-associated nephrotoxicity.
- Group **V1C0**: vancomycin overexposure without associated nephrotoxicity.
- Group **V1C1:** both vancomycin overexposure and associated nephrotoxicity.

Alongside the 25 patients in **V0C1** and **V1C1** groups, 50 patients in both **V0C0** and **V1C0** were randomly selected. We recorded demographic characteristics, comorbid conditions, pathogens involved, sites of infection, clinical circumstances leading to vancomycin therapy and exposure to other known potential nephrotoxic therapy. The detailed list of recorded items is shown in appendix 1. The chronological course of vancomycin therapy was rebuilt for every patient, with time of onset and interruption of

therapy, detailed vancomycin dosage, time of vancomycin trough or peak concentration measurements (including time since the last vancomycin administration), time of creatinine measurements and variations of creatinine levels from baseline. These data allowed to further calculating various parameters for every patient, such as average or maximal vancomycin doses per day; cumulated doses administrated over therapy, average and maximal trough or peak-concentrations observed or minimal and average and maximal creatinine levels. These data were subject in another analysis to population pharmacokinetic modelling, allowing to retrieve the maximum likelihood value of some pharmacokinetic parameters for vancomycin (27). The patients' glomerular filtration rate was estimated as well using Cockcroft-gault and MDRD formulas[1]. All of the recorded data were then investigated as potential predictors of vancomycin-associated nephrotoxicity or vancomycin overexposure.

2. Group population description

A description of the four *reviewed* groups was first conducted. Summary tables were used to describe demographic characteristics and other data mentioned above. Chronological course of vancomycin therapy was graphically represented for every patient.

3. Relationship between vancomycin overexposure and associated nephrotoxicity.

The association between vancomycin overexposure and vancomycin-induced nephrotoxicity was assessed using a Chi-square test as the main working hypothesis of the study. It was performed on both screened groups population and reviewed groups' population, although only the result on the reviewed population was considered. The reason is that the screened population contains a number of "uncertain" patients dropped out for not matching the **C1** criterion. These patients were completely dropped out of the analysis and not included in the C0 groups, to avoid contamination by a possibly higher number of patients with renal problems related or not to vancomycin, yet not corresponding to our definition of vancomycin-associated nephrotoxicity.

[1] Cockcroft-gault : creatinine clearance = ((140 - age in years) x (weight in kg) x *K*) / (serum creatinine in µmol/l), where *K* is 1.23 for men and 1,04 for women
MDRD: eGFR = 186 x (creatinine in µmol/l x $0{,}0113^{)-1{,}154}$ x $age^{-0{,}203}$ (x 0.742 for women or 1.21 african-american)

4. Assessment of hypothesized risk factors for vancomycin-associated nephrotoxicity.

Hypothesized risk factors for vancomycin-associated nephrotoxicity were assessed using a logistic regression approach. Results were contrasted between a non-nephrotoxic group (**V0C0 + V1C0** patients) and a nephrotoxicity group (**V0C1 + V1C1** patients), regardless of the level of vancomycin exposure. As the sample size of our *reviewed* groups had been artificially limited to 25 or 50, the group samples had to be weighted for the Chi-square test in order to represent their actual abundance in the original population of patients receiving vancomycin. We used the "sampling weight" option in STATA, i.e. a weighting factor proportional to the inverse of the probability that the patient is included in the analysis due to sampling strategy. This type of weighting is assumed not to unduly affect the significance levels. . Beyond vancomycin overexposure (main working hypothesis), all other potential risk factors for nephrotoxicity listed in appendix 1were first evaluated using univariate analyses. Some associations were excluded as they were evidently constructed. Multivariate analysis was then performed for confirmation, first without and then with inclusion of vancomycin overexposure as risk factor. A stepwise approach was used to select variables to include in the multivariate model.

5. Assessment of hypothesized risk factors for vancomycin overexposure.

Hypothesized risk factors for vancomycin overexposure were also assessed using a weighted logistic regression approach. In this analysis, results were contrasted between a non-overexposed group (**V0C0 + V0C1** patients) and an overexposed group (**V1C0 + V1C1** patients). Here again, the weighting of group samples was required to represent their frequency in the original population. Vancomycin-associated nephrotoxicity as risk factor for vancomycin exposure (main working hypothesis) was evaluated, and univariate analyses were then used to test associations between vancomycin overexposure and other potential risk factors listed in appendix 1. Some associations were excluded due to constructed correlation. A stepwise multivariate confirmatory analysis was then performed, first without and then with inclusion of induced nephrotoxicity as risk factor.

6. Temporal link between vancomycin and creatinine changes.

An exploration of the temporal link between vancomycin trough-concentrations and creatinine variations was attempted, keeping only the values of vancomycin trough-concentrations measured on the same day as a creatinine value. Spearman's rank correlation was computed to assess the association between same day vancomycin and creatinine values. Cross correlations with Spearman's method were then used to assess the associations between given vancomycin values and anterior or posterior creatinine levels (up to 3 lag shifts each). Similarly, cross-associations were determined between

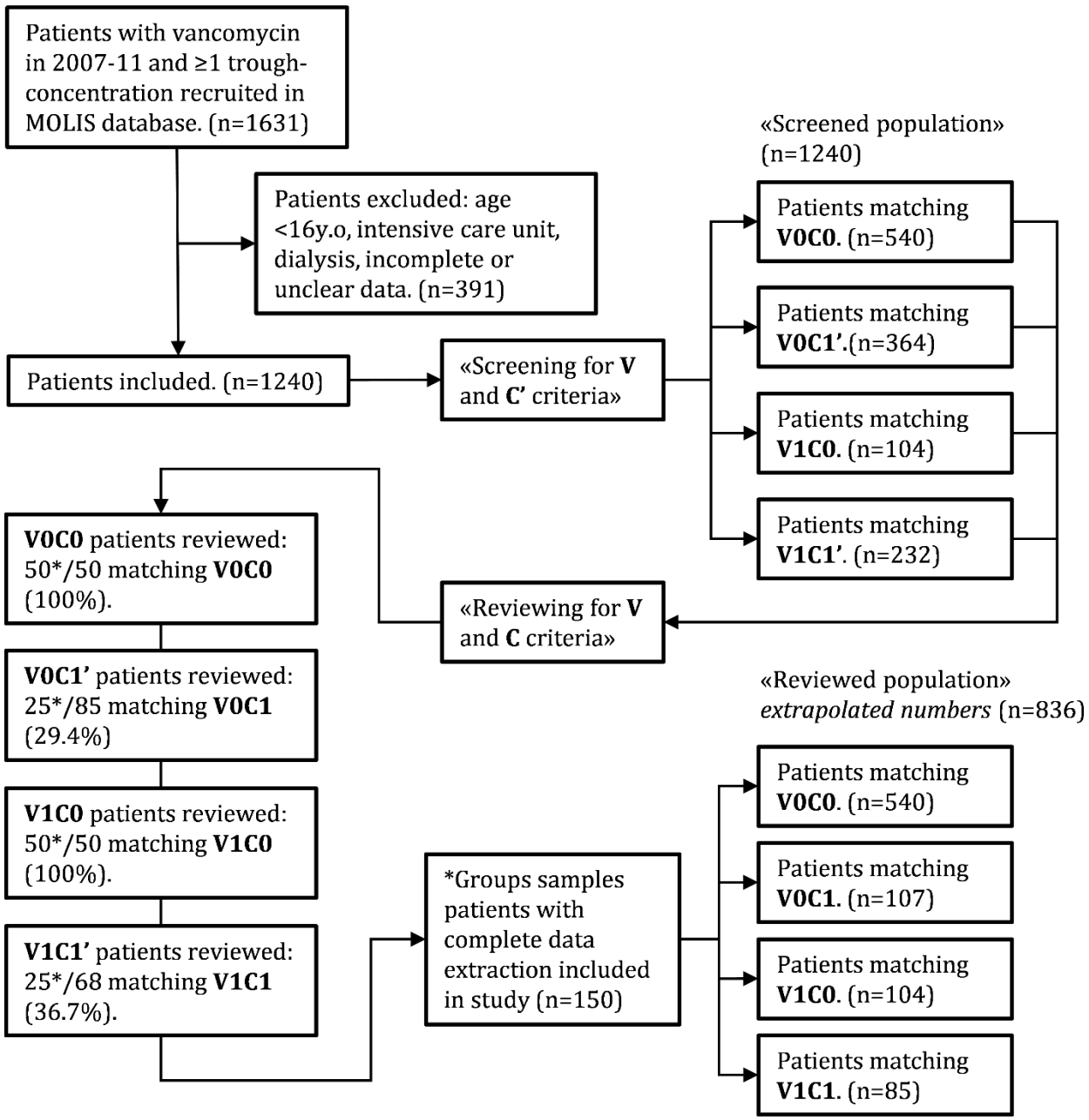
vancomycin dosages and trough-concentrations, both simultaneous and lagged in the past or in the future (up to 3 lag shifts each). We also assessed similarly the cross-associations between vancomycin dosage and creatinine levels. This determination of precedence in trends has been advocated to evaluate the direction of potential causality relationships between multiple longitudinal variables. Finally, we applied to the series of vancomycin and creatinine values a formal Granger causality test, aiming to determine whether one time series is able to forecast another one better than the mere previous values of the second one.

All statistical analyses were performed using the STATA software (version 13.1, StataCorp, 4905 Lakeway Drive, College Station, Texas 77845 USA). Test significance throughout our analyses was considered for *p* values smaller than 0.05 ($P<0.05$).

Results

1. Descriptive analysis

1.1 Study population



- Extrapolated number of patients in reviewed population example: 25 out of 85 patients (=29.4%) from **V0C1'** group (n=364) matching **C1** criteria when reviewed. Total number of patients matching **V0C1** in reviewed population is so defined as 29.4% of **V0C1'** screened population, i.e 29.4% from 364 = 107 patients.

Figure 2: Flow chart depicting the selection process of patients included in the study.

1240 patients were included from the hospital laboratory database, composing the *screened* population. 150 patients were randomly selected from the screened population groups and included in the full analysis after extensive review of their medical records: 50 from **V0C0**, 25 from **V0C1**, 50 from **V1C0** and 25 from **V1C1**. During the reviewing process, 404 patients matching the **C1'** criterion were dropped out of the analysis as they didn't truly match the definition of vancomycin-associated nephrotoxicity as earlier defined (i.e. **C1** criterion). The patients reviewed and kept in the analysis were used to calculate and extrapolate the frequency of true **V0C1** and **V1C1** cases in the whole study population. At the end, 836 patients were considered as truly matching **V** and **C** criteria, composing the *reviewed* population. Thus, each of the 50 patients extensively reviewed among the 540 assigned in group **V0C0** was given a weight of 540/50=10.8 during further analyses; each of the 25 reviewed among the 107 in group **V0C1** was weighted 4.28; each of the 50 reviewed among 104 in group **V1C0** was weighted 2.08; and each of the 25 reviewed among 85 in group **V1C1** was weighted 3.40. These "sampling weights" represent the inverse of the probability that each patient was included in the analysis considering the sampling strategy, and are used to compute association coefficients corrected for sampling inequity, without unduly affecting the significance levels.

Demographic characteristics and clinical data extracted from hospital medical records are summarize for every sample groups in appendix 2. The mean age (± SD) was quite similar between groups, ranging from 63.3 (±16.4) to 69.8 (±13.7) years. In **V0C0, V0C1** and **V1C0** groups, the sex ratio male/female was close to 60/40%, but was 80/20% in **V1C1**. The most frequent comorbid conditions were arterial hypertension and diabetes. Arterial hypertension occurred approximately 1.5 times more often in **V1C0** than in the other groups (60 *vs* 38-44%). Pre-existent heart failure was more than two times more prevalent in **V1C1** (52 *vs* 18-20%). Skin and soft tissues infections were predominant in **V0C0 and V1C0** groups (42 and 38% *vs* 20 and 24% in the other groups). **V1C1** patients suffered twice more than others from bloodstream infection (44 *vs* 16-18%).

MRSA and *Staphylococcus epidermidis* were the most frequent pathogens found in every group, with 22, 32, 38 and 56% in **V0C0, V0C1, V1C0** and **V1C1** groups respectively for MRSA and 30, 24, 20 and 28% respectively for *S. epidermidis.* Vancomycin therapy was mostly started in a febrile state context or close from a surgical act complicated with an infection requiring vancomycin. 96% of patients in whom nephrotoxicity occurred (**V0C1** and **V1C1**) received at least one other potentially nephrotoxic treatment (and/or causing pre-renal dysfunction) during vancomycin therapy, when only 64 and 74% received one in **V0C0** and **V1C0** respectively. Diuretics and NSAIDs were the most frequent treatments administrated. It is interesting to note a marked difference between groups regarding aminoglycosides and anti-fungal agents, far more prevalent in **V0C1** and **V1C1.**

Patients received vancomycin for an average of 14.2 (±10.5) days in **V0C0**, 14.9 (±10.5) days in **V0C1**, 15.2 (±10.6) days in **V1C0** but 24 (±16) days in **V1C1.** The mean daily dose was quite similar, ranging from 1.2 – 1.6°g. The average cumulated dose received was again quite similar for **V0C0, V0C1** and **V1C0** groups with 22.4 (±19.5)°g, 22.0 (±17.5)°g, 23.1 (±18.05)°g respectively but higher in **V1C1** with 30.9 (±25.3)°g.

1.2. Relationship between vancomycin-associated nephrotoxicity and overexposure.

In the reviewed population, the global incidence of vancomycin-associated nephrotoxicity was estimated to be 23% (192 out of 836 patients). More specifically, according to our definition, nephrotoxicity occurred in 16.5% of patients without vancomycin overexposure (107 out of 647 patients) *versus* 45% of patients with vancomycin overexposure observed (85 out of 189). A Chi-square test confirmed vancomycin-induced nephrotoxicity to be significantly associated with vancomycin overexposure, with an odds ratio of 4.12 ([95%CI] = 2.85 – 5.96, p <0.001). This result is in line with our main working hypothesis.

2. Assessment of hypothesized risk factors.

The variables retrieved from patient records were assessed as hypothesized risk factors for vancomycin-induced nephrotoxicity. They were contrasted between a non-nephrotoxic group (**V0C0** + **V1C0**, n=100) and a nephrotoxic group (**V0C1** + **V1C1**, n=50) as defined earlier. The same process was applied thereafter to assess risk factors for vancomycin overexposure. Variables contrasted according to both these classifications are summarized in the table below, with the most frequent comorbid conditions, pathogens involved, sites of infection, clinical conditions and nephrotoxic co-treatment put in bold.

Variables	Non-nephrotoxic group (n=100)	Nephrotoxic group (n=50)	Non-overexposed group (n=75)	Overexposed group (n=75)
Population characteristics				
Age, mean years (±SD)	66.6 (±15.4)	67.8 (±13.9)	64.2 (±15.4)	69.8 (±13.9)
Sex %male (% female)	60 (40)	70 (30)	61.3 (38.7)	65.3 (34.7)
Body weight, mean kg (±SD)	74.9 (±17.2)	81.02 (±17.07)	78.8 (±17.2)	75.11 (±17.3)
Obesity (%)	22 (22)	16 (32)	21.0 (28.0)	17.0 (22.7)
Comorbid conditions				
Arterial hypertension (%)	**49 (49)**	**21 (42)**	**30 (40)**	**40 (53.3)**
Pre-existent renal failure (%)	19 (19)	18 (36)	14 (18.7)	23 (30.7)
Heart failure (%)	21 (21)	14 (28)	9 (12)	26 (34.7)
Diabetes mellitus (%)	31 (31)	13 (26)	22 (29.3)	22 (29.3)

Pathogens				
MSSA (%)	4 (4)	2 (4)	4 (5.3)	2 (2.7)
MRSA (%)	**30 (30)**	**22 (44)**	19 (25.3)	**33 (44)**
S. Epidermidis (%)	25 (25)	13 (26)	**21 (28)**	17 (22.7)
Streptococcus species (%)	6 (6)	1 (2)	5 (6.7)	2 (2.7)
Enterococcus species (%)	19 (19)	7 (14)	11 (14.7)	15 (20)
Unidentified pathogen (%)	14 (14)	7 (14)	14 (18.7)	7 (9.3)
Other (%)	6 (6)	6 (12)	6 (8)	6 (8)
Site of infection				
Bloodstream (%)	18 (18)	**15 (30)**	13 (17.3)	20 (26.7)
Bone (%)	10 (10)	4 (8)	6 (8)	8 (10.7)
Prosthetics (%)	13 (13)	5 (10)	8 (10.7)	10 (13.3)
Skin and soft tissues (%)	**40 (40)**	11 (22)	**27 (36)**	**24 (32)**
Endocarditis (%)	2 (2)	3 (6)	4 (5.3)	1 (1.3)
Pneumonia (%)	2 (2)	3 (6)	3 (4)	2 (2.7)
Meningitis (%)	4 (4)	0 (0)	3 (4)	1 (1.3)
Intra-abdominal (%)	1 (1)	2 (4)	2 (2.7)	3 (4)
Other (%)	14 (14)	7 (14)	9 (12)	6 (8)
Clinical conditions				
Surgery (%)	**40 (40)**	16 (32)	**26 (34.7)**	**30 (40)**
Febrile neutropenia (%)	5 (5)	3(6)	6 (8)	2 (2.7)
Fever (%)	30 (30)	**17 (34)**	25 (33.3)	22 (29.3)
Septic shock (%)	3 (3)	4 (8)	3 (4)	4 (5.3)
Malignancy (%)	1 (1)	1 (2)	1 (1.3)	1 (1.3)
Other (%)	21 (21)	9 (18)	14 (18.7)	16 (21.3)
Concomitant exposure to				
Nephrotoxic co-treatment, mean number (±SD)	1.15 (±1.01)	2.04 (±1.05)	1.4 (±1.13)	1.6 (±1.07)
Any nephrotoxic co-treatment (%)	69 (69)	48 (96)	56 (74.7)	61 (81.3)
- Inc. aminoglycosides (%)	2 (2)	7 (14)	5 (6.7)	4 (5.3)
- Inc. diuretics (%)	31 (31)	**35 (70)**	**34 (45.3)**	32 (42.7)
- Inc. antifungal agent (%)	1 (1)	8 (16)	5 (6.7)	4 (5.3)
- Inc. NSAIDs (%)	**53 (53)**	25 (50)	30 (40)	**48 (64)**
- Inc. ACE inhibitor (%)	23 (23)	18 (36)	21 (28)	20 (26.7)
- Other (%)	5 (5)	8 (16)	6 (8)	8 (10.7)
Vancomycin parameters				
Mean duration, days (±SD)	14.7 (±10.5)	19.5 (±14.15)	14.4 (±10.4)	18.15 (±13.2)
Mean trough conc., mg/L (±SD)	15.5 (±5.3)	16.2 (±5.3)	*12.0 (±3.3)*	*19.5 (±4.12)*
Mean peak conc., mg/L (±SD)	23.6 (±6.4)	27.3 (±15.7)	22.8 (±5.14)	27.02 (±13.8)
Mean daily dose, g (±SD)	1.6 (±0.6)	1.3 (±0.6)	1.51 (±0.5)	1.5 (±0.7)
Mean total dose, g (±SD)	22.7 (±18.7)	26.4 (±22.01)	22.3 (±18.8)	25.7 (±20.9)
Mean Vancomycin clearance (±SD)	3.8 (±1.8)	3.06 (±1.6)	4.3 (±1.9)	2.9 (±1.3)

Volume of distribution (±SD)	126.3 (±41.4)	155.7 (±79.4)	151.1 (±63.9)	121.1 (±48.3)
Creatinine parameters				
Mean sCr, µmol/L (±SD)	**94.7 (±105.5)*	**119.6 (±60.5)*	89.5 (±40.6)	116.5 (±124.8)
Max sCr, µmol/L (±SD)	**106.5 (±114.2)*	**214.4 (±140)*	118.04 (±70.15)	166.9 (±171.8)
Mean maximal sCr variation from baseline in %	**9.2*	**140.7*	45.3	60.8
Estimation of renal function				
eClCr with Cockcroft-Gault formula, mL/min (±SD)	**85.4 (±33.03)*	**82.4 (±50.9)*	93.4 (±45.5)	75.2 (±30.7)
eGFR with MDRD formula, mL/min/1.73m² (±SD)	**90.18 (±31.6)*	**80.8 (±39.0)*	90.6 (±35.8)	83.4 (±32.8)

sCr: serum creatinine; eClCr: estimated renal clearance of creatinine; eGFR: estimated glomerular filtration rate.
**constructed outcome.*

Table 1: Variables contrasted for non-nephrotoxic *vs* nephrotoxic groups, and for non-overexposed *vs* overexposed groups.

2.1. Risk factors for vancomycin-associated nephrotoxicity.

Contrasting nephrotoxic group *vs* non-nephrotoxic group, the mean age was 67.8 (±13.9) *vs* 66.6 (±15.4) years. 70 *vs* 60% were male, with a mean body weight of 81.02 (± 17.1) *vs* 74.9 (± 17.2) kg. The most frequent comorbid condition was arterial hypertension in both groups (42 *vs* 49%). The majority of patients were treated for bloodstream infection (30%) *vs*. skin and soft tissues infection (42%). MRSA was in both cases the most frequent pathogens involved. Regarding clinical conditions, septic shock occurred almost three times more often in nephrotoxic group (8 *vs* 3%). 96 *vs* 69% of patients received a nephrotoxic co-treatment, with aminoglycosides and antifungal agents far more frequent in nephrotoxic group (14 *vs* 2% and 16 *vs* 1% respectively). Patients in nephrotoxic group were treated for a longer period (19.5 *vs* 14.7 days), but received smaller daily doses (1.3 *vs* 1.6 g). Nevertheless, the cumulative dose received over therapy was higher in the nephrotoxic group (26.4 *vs* 22.7 g). Creatinine parameters and estimation of renal function results differed, but this association is clearly constructed, as resulting from groups definition.

Variables	Univariate OR (95% CI)	P value	Multivariate OR (95% CI)	P value
Population characteristics				
Body weight	1.02 (0.99-1.04)	0.06		
Comorbid conditions				
Pre-existent renal failure	2.3 (1.0-5.4)	0.05		
Heart failure	3.12 (1.3-7.6)	0.012		
Pathogens				
MRSA	2.3 (1.05-5.0)	0.04		
Site of infection				
Bloodstream	1.8 (0.8-4.3)	0.2		

Skin and soft tissues	0.41 (0.2-0.9)	0.03		
Intra-abdominal	14.4 (1.3-164)	0.03	30.2 (1.15-793)	0.04
Clinical conditions				
Septic shock	3.7 (0.6-22)	0.16		
Concomitant exposure(CE) to				
any nephrotoxic treatment*	12.6 (2.8-57)	0.001		
aminoglycosides	8.13 (1.2-53)	0.03	10.9 (1.9-60)	0.006
diuretics	5.5 (2.4-12)	<0.001	6.3 (2.8-14)	<0.001
antifungal agent	11.2 (1.3-93)	0.03		
ACE inhibitors	2.0 (0.9-4.5)	0.1		
Number of CE	2.4 (1.7-3.4)	<0.001		
Therapy course				
Vancomycin overexposure	4.12 (2.09-8.15)	<0.001	4.8 (2.14-10.7)	<0.001
Duration of therapy	1.03 (1.0-1.07)	0.05		
Mean dose per day	0.99 (0.99-1.00)	0.08		
Mean trough concentration	1.09 (1.016-1.18)	0.015		
Pharmacokinetics parameters				
Mean Vancomycin clearance	0.7 (0.6-0.9)	0.002		
Vancomycin volume of distribution	1.01 (1.00-1.02)	0.008		

*from treatments retrieved for the study.
Only variables with p value inferior to 0.2 are shown here

Table 2: Results of univariate and multivariate analysis for vancomycin-associated nephrotoxicity.

After univariate analysis, heart failure, MRSA, skin and soft tissues infection, intra-abdominal infection, septic shock and concomitant exposure to aminoglycosides, diuretics or antifungal agents are found to be significantly associated with vancomycin-induced nephrotoxicity. Odds ratio, 95% confidence interval and p values for these factors are shown in table 2. Vancomycin overexposure is of course again associated with nephrotoxicity (OR 4.12, [95%CI] 2.09-8.15, p<0.001). Duration of therapy is barely significant (OR 1.03, [95%CI] 1-1.07, p = 0.05). Interestingly, variables such as age, sex, obesity, vancomycin daily and total doses were not found to be risk factors. Detailed univariate analysis with Odds ratio, 95% CI and p values are shown in appendix 3.

In the multivariate analysis, vancomycin-induced nephrotoxicity remained associated with vancomycin overexposure (OR 4.8, p<0.001), aminoglycosides (OR 10.9, p=0.006), diuretics (OR 6.3, p<0.001) and intra-abdominal infections (OR 30.2, p=0.04). Conversely, inclusion of vancomycin overexposure as a risk factor made MRSA and antifungal agents to be dropped out from the list of predictors in the multivariate analysis.

2.2. Risk factors for vancomycin overexposure.

Contrasting overexposed group *vs* non-overexposed group, the mean age was 69.8 (±13.9) *vs* 64.2 (±15.4) years. 65.3 *vs* 61.3% were male, with a mean body weight of 75.11 (± 17.3) *vs* 78.8 (±17.2) kg. While the most frequent comorbid condition was arterial hypertension in both groups, it is interesting to note the marked difference for pre-existent renal failure (30.7 *vs* 18.7%) and heart failure (34.7 *vs* 12%). The majority of patients were treated for skin and soft tissues infection in both groups, with bloodstream infection being more frequent in the overexposed group (26.7 *vs* 17.3%). MRSA was in both cases the most frequent pathogens involved (44 *vs* 25.3%). 81.3 *vs* 71.7% of patients received a nephrotoxic co-treatment, with NSAIDs being quite more frequent in overexposed group (64 *vs* 40%). Patients in overexposed group were treated for a longer period (18.15 *vs* 14.4 days), daily dose being similar (1.5 g/L in both groups) but cumulative dose received over therapy being higher (25.7 *vs* 22.3 g/L). Mean creatinine levels were globally higher in overexposed group (116.5 *vs* 89.5 µmol/L), alongside an apparent impaired renal function estimation using either Cockcroft-Gault or MDRD formula.

Variables	Univariate OR (95% CI)	P value	Multivariate OR (95% CI)	P value
Population characteristics				
Age	1.02 (1.004-1.05)	0.03		
Comorbid conditions				
Arterial hypertension	1.6 (0.8-3.2)	0.16		
Pre-existent renal failure	2.3 (1.05-5.2)	0.04	2.5 (1.12-5.5)	0.03
Heart failure	5.3 (2.12-13)	<0.001	8.13 (2.6-25.2)	<0.001
Pathogens				
MRSA	2.8 (1.3-5.7)	0.007		
Streptococcus species	0.3 (0.05-1.6)	0.15		
Site of infection				
Bloodstream	2.0 (0.9-4.5)	0.11		
Meningitis	0.2 (0.02-2.01)	0.18		
Concomitant exposure(CE) to				
Number of CE	1.5 (1.08-2)	0.014	0.5 (0.3-0.9)	0.02
any nephrotoxic treatment*	2.3 (1.04-5.1)	0.04		
diuretics	1.4 (0.7-2.8)	0.3		
NSAIDs	2.5 (1.3-5.1)	0.008	5.4 (2.2-13.5)	<0.001
Therapy course				
Mean duration	1.03 (1.004-1.07)	0.03		
Mean peak concentration	1.06 (1.0-1.14)	0.08		
Renal function				

Vancomycin-associated nephrotoxicity	4.12 (2.09-8.15)	<0.001	5.2 (2.3-11.9)	<0.001
Mean Creatinine level	1.01 (1.002-1.02)	0.011		
Max Creatinine level	1.008 (1.004-1.01)	<0.001		
eClCr with Cockcroft-Gault formula, mL/min	0.98 (0.97-0.99)	0.004		
eGFR with MDRD formula, mL/min/1.73m²	0.99 (0.98-1.002)	0.11		

*from treatments retrieved for the study.
Only variables with p value inferior to 0.2 are shown here

Table 3: Results of univariate and multivariate analysis for vancomycin overexposure.

After univariate analysis, heart failure, MRSA, skin and soft tissues infection, intra-abdominal infection, septic shock and concomitant exposure to aminoglycosides, diuretics or antifungal agents are found to be significantly associated with vancomycin-induced nephrotoxicity. Odds ratio, 95% confidence interval and p values for these factors are shown in table 3. Induced nephrotoxicity was evidently again associated with overexposure (OR 4.12, [95%CI] 2.09-8.15, p<0.001). Duration of therapy is barely significant (OR 1.03, [95%CI] 1-1.07, p = 0.05). Again, variables such as body weight, sex, obesity, vancomycin daily and total doses were not found to be risk factors. Detailed univariate analysis with odds ratio, 95% CI and p values are shown in appendix 4.

In the multivariate analysis, vancomycin overexposure was associated with vancomycin-associated nephrotoxicity (OR 5.2, p<0.001) along with NSAIDs (OR 5.4, p<0.001), pre-existent renal failure (OR 2.5, p=0.03) and heart failure (OR 8.13, p<0.001). Surprisingly, an odds ratio <1 was found for the number of concomitant exposures to other nephrotoxic treatments (OR 0.5, p=0.02). Inclusion of nephrotoxicity as a risk factor for overexposure led to drop out MRSA from the multivariate analysis. It is here interesting to note that renal function variables, especially the estimated creatinine clearance with Cockcroft-Gault equation, mean and maximal creatinine levels, outshined vancomycin-associated nephrotoxicity association with overexposure (OR 2.41, p>0.2) when included in the multivariate model! But considering that these variables are directly influenced by vancomycin overexposure, although they contribute to its occurrence (vicious circle effect), they were left out the final multivariate model.

3. Temporal link between vancomycin and creatinine changes.

3.1. Cross correlations approach

As explained earlier, only vancomycin trough-concentrations with a creatinine sampled the same day were kept for this exploration. First, a simple rank-based correlation found vancomycin and creatinine to be positively correlated (Spearman's rho R = 0.3, p value <0.001). This is another indication of

association between induced-nephrotoxicity and overexposure (creatinine levels tend to rise when trough-concentration are high). Variables were then created by shifting creatinine values up to 3 lags in the past (sCr -1, sCr -2 and sCr -3) and in the future (sCr +1, sCr +2, sCr +3). Correlation between a trough-concentration and creatinine values from the past and the future were then assessed using Spearman's rank correlation coefficient (R).

Variables		*VTr*	*sCr*	*sCr -1*	*sCr -2*	*sCr -3*	*sCr +1*	*sCr +2*	*sCr +3*
VTr	R	1							
	p								
sCr	R	0.3	1						
	p	***<0.001***							
sCr -1	R	0.06	0.9	1					
	p	*0.4*	***<0.001***						
sCr -2	R	0.14	0.7	0.8	1				
	p	*0.2*	***<0.001***	***<0.001***					
SCr -3	R	0.15	0.6	0.6	0.8	1			
	p	*0.3*	***<0.001***	***<0.001***	***<0.001***				
sCr +1	R	0.4	0.9	0.7	0.6	0.3	1		
	p	***<0.001***	***<0.001***	***<0.001***	***<0.001***	*0.1*			
sCr +2	R	0.4	0.7	0.6	0.3	0.3	0.9	1	
	p	***<0.001***	***<0.001***	***<0.001***	*0.1*	*0.1*	***<0.001***		
sCr +3	R	0.4	0.6	0.3	0.3	0.5	0.7	0.9	1
	p	***<0.001***	***<0.001***	*0.1*	*0.1*	*0.1*	***<0.001***	***<0.001***	

sCr: serum creatinine; VTr: vancomycin trough-concentration.
Significant correlations are shown in bold.

Table 4: Cross correlations matrix between present vancomycin trough-concentration and creatinine levels through time (from 3 shifts in the past to 3 shifts in the future).

Cross correlations results are presented in table 4. Vancomycin trough-concentration is significantly correlated with creatinine levels of the *future* (R = 0.4, p<0.001 for 1, 2 and 3 shifts), but less with levels from the past. Creatinine is strongly auto-correlated with both levels from the past and the future (with R values decreasing when shift increases). The same approach was applied to test cross-correlations between creatinine and past/future trough-concentrations, with results shown in appendix 5. Creatinine level is positively correlated with trough-concentrations in the *past* (R = 0.4, p<0.001 for 1 and 2 shifts; R = 0.4, p = 0.003 for 3 shifts). Vancomycin seems to be less auto-correlated as creatinine (p<0.001 only for 1 shift in the past and in the future).

Cross correlations were also performed between vancomycin current dosage and past/future trough-concentrations (table 5). Interestingly, dosage is not correlated with simultaneous trough-concentration but positively correlated with the *future* ones (R = 0.3, 0.3 and 0.3 with p <0.001, 0.02 and 0.011 for 1, 2 and 3 shifts respectively) and negatively with the past ones (R = -0.4, -0.4 and -0.3 with p <0.001, <0.001 and 0.05 for 1, 2 and 3 shifts respectively).

Variables		*Dose*	*VTr*	*VTr -1*	*VTr -2*	*VTr -3*	*VTr +1*	*VTr +2*	*VTr +3*
Dose	R	1							
	p								
VTr	R	0.1	1						
	p	*0.09*							
VTr -1	R	- 0.4	0.3	1					
	p	***<0.001***	***<0.001***						
VTr -2	R	- 0.4	- 0.03	0.4	1				
	p	***<0.001***	*0.8*	***<0.001***					
VTr -3	R	- 0.3	0.04	0.02	0.4	1			
	p	*0.05*	*0.8*	*0.9*	***0.014***				
VTr +1	R	0.3	0.3	- 0.03	0.04	- 0.09	1		
	p	***<0.001***	***<0.001***	*0.8*	*0.8*	*0.7*			
VTr +2	R	0.3	- 0.03	0.04	- 0.09	0.2	0.3	1	
	p	***0.02***	*0.8*	*0.8*	*0.7*	*0.3*	***0.003***		
VTr +3	R	0.3	0.04	- 0.09	0.2	0.09	0.2	0.5	1
	p	***0.011***	*0.8*	*0.7*	*0.3*	*0.8*	*0.09*	***<0.001***	

VTr: vancomycin trough-concentration.
Significant correlations are shown in bold

Table 5: Cross correlations matrix between present vancomycin dose and trough concentrations through time (from 3 shifts in the past to 3 shifts in the future).

Finally, cross correlations between current dose and past/future creatinine levels finds dose *negatively* correlated with every shifts of creatinine either in the past or in the future (p <0.05 for all shifts). Detailed results are shown in appendix 6.

3.2. Granger causality test approach.

Granger causality test was used to evaluate how vancomycin and creatinine act on one another. Prediction of future trough-concentrations based on past ones somewhat improved when 1 shift of past creatinine level was included in the model already including past vancomycin levels, though it was not significant. (Likelihood ratio for model improvement: LR 3.06, p = 0.08). When 3 shifts of past

creatinine levels were included, prediction failed to be better (LR 0.29, p = 0.96). Reciprocally, prediction of future creatinine levels based on past ones was enhanced when 1 shift of vancomycin trough concentration was included in the model based on past creatinine values, though it was again not significant (LR 3.12, p = 0.08). When 3 shifts were included, prediction failed to better (LR 0.35, p = 0.95).

4. Illustrative cases.

Fig. 2-5: Clinical courses showing vancomycin levels with daily doses *(g/d)*, and creatinine (sCr) levels over days.

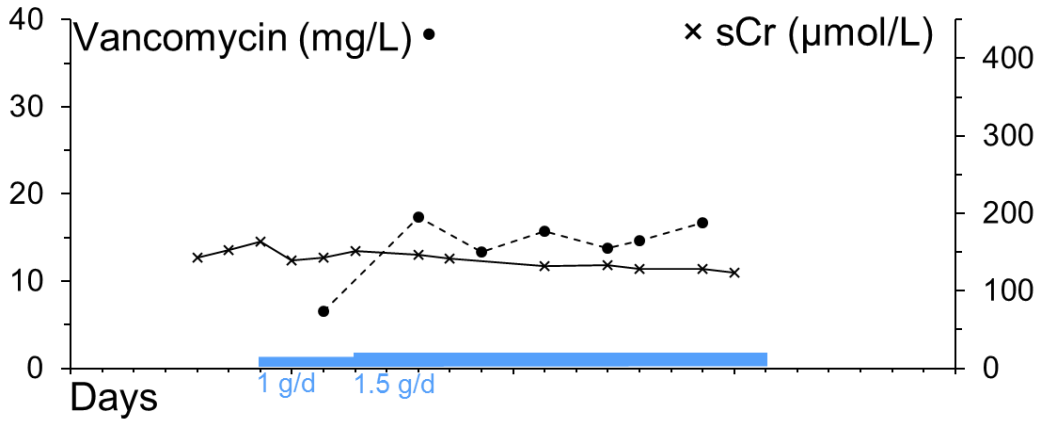


Fig.2: V0C0 male patient, 38 years old, 69kg, treated for a *S. epidermidis* bloodstream infection. Target trough was maintained all along therapy.

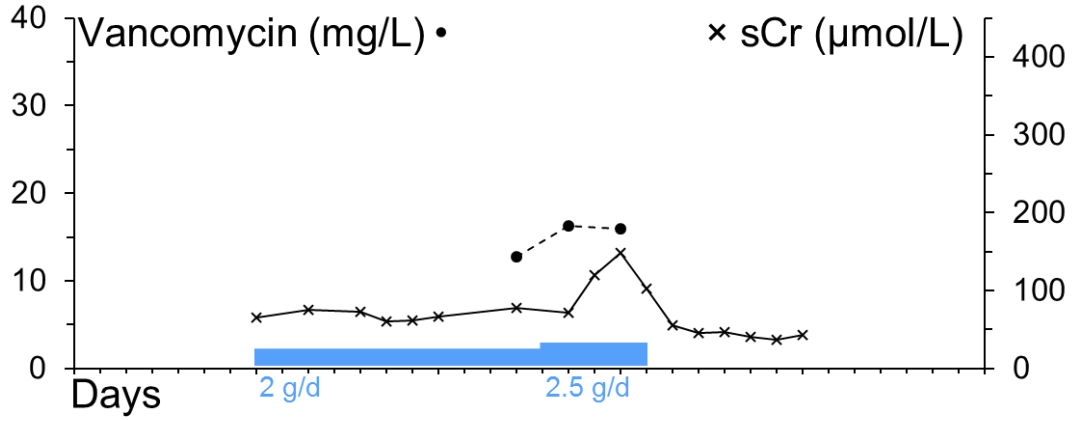


Fig.3: V0C1 male patient, 50 years old, 86kg, known for a heart failure, treated for a MRSA bloodstream infection and taking diuretics, NSAIDs and ACE inhibitors as co-treatment. A maximal 129% rise from baseline in creatinine levels occurred shortly after dosage intensification of vancomycin.

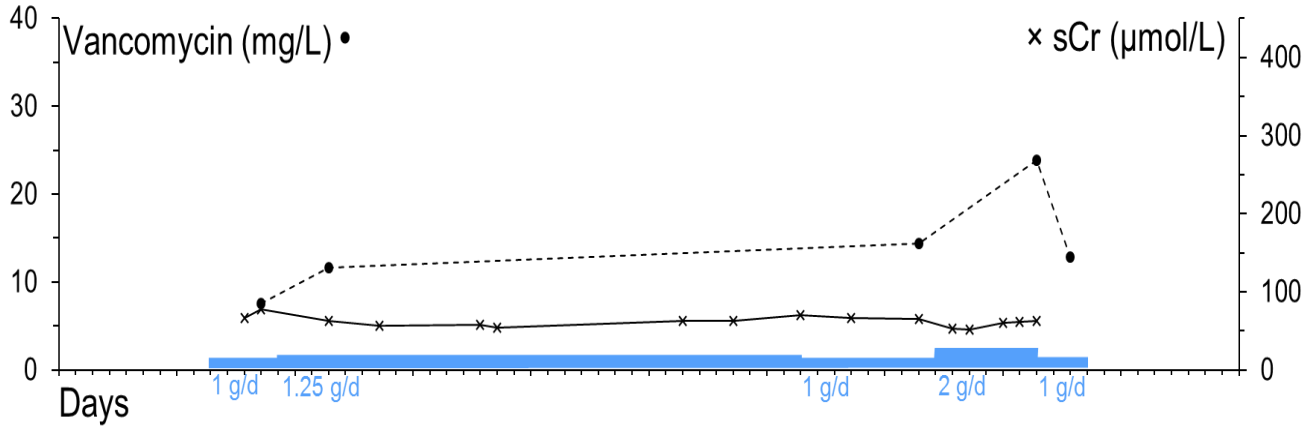


Fig.4: V1C0 female patient, 78 years old, 69kg, known for a heart failure with a *S. epidermidis* acute prosthetic joint infection. During five days, she received both aminoglycosides and vancomycin. She presented one trough-concentration 23.9 mg/L, leading to immediate dosage adaptation.

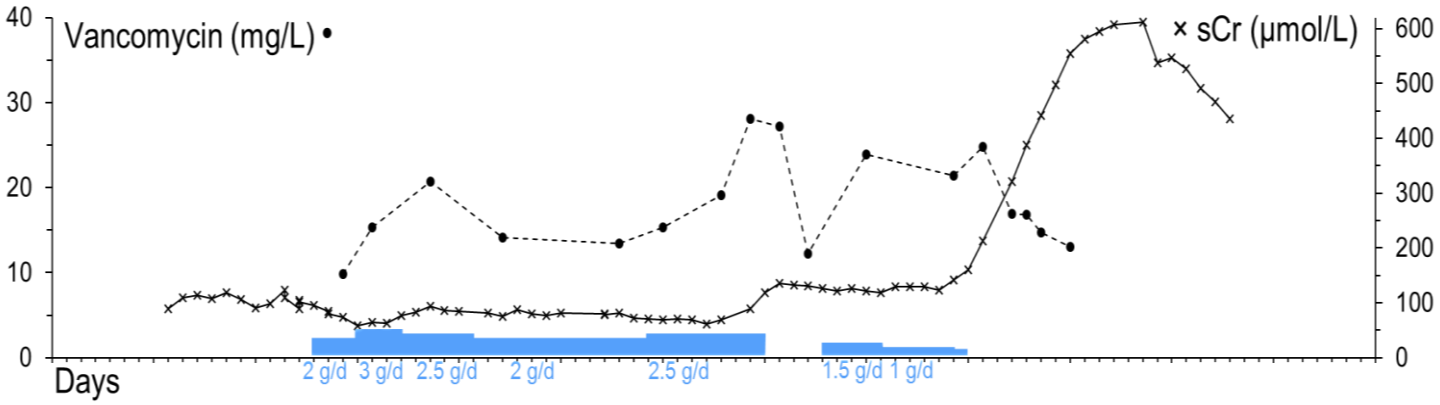


Fig.5: V1C1 male patient, 60 years old, 110 kg, known for obesity, arterial hypertension, diabetes and heart failure, who received vancomycin for a severe MRSA osteomyelitis requiring high doses of vancomycin to ensure therapeutic effect. After a first disruption due to overexposure, vancomycin was started again and kept on despite 3 new consecutive trough-concentrations >20mg/L. Nephrotoxicity occurred some days later, persisting despite vancomycin disruption. The patient was then found to suffer from acute tubular necrosis.

Discussion

This retrospective observational study recorded extensive medical data for 150 patients, randomly sampled among a population of 836 patients matching our inclusion criteria, who received a vancomycin therapy with therapeutic drug monitoring (TDM) surveillance. Chronological courses of vancomycin therapy were rebuilt for each patient of the reviewed sample in order to describe precisely the variations in vancomycin and creatinine levels along time.

A clear association was confirmed between vancomycin overexposure and occurrence of vancomycin-induced nephrotoxicity (OR 4.12, p <0.001). This association was robust and persisted without important changes when other risk factors were included in multivariate predictive models. The frequency of nephrotoxicity occurrence in patients meeting overexposure criteria (45% versus 16.5% without overexposure) is compatible with other observations in the literature. Among our study patients, nephrotoxic episodes were quickly reversible without persistent renal damages in most cases, except in three cases where patients developed acute tubular necrosis.

Regarding vancomycin-associated nephrotoxicity, it is interesting to note that none of age, sex, body weight or obesity were found to be risk factors. Univariate analysis shows however body weight to be associated with a 20% risk increase for every 10 kg. If duration of therapy and total dose received are longer and higher in the nephrotoxic group, the mean daily dose of vancomycin is lower, probably reflecting dosage adaptation for patients with kidney function impairment. According to our logistic regression analysis, renal aggravating factors such as concomitant treatment with aminoglycosides and diuretics were found to significantly add to the risk of vancomycin-associated nephrotoxicity. Abdominal infections are strongly associated with nephrotoxicity, but this result is based on little number of patients. Due to associations with vancomycin overexposure, some factors identified by univariate analysis, such as MRSA infection, were left out of the multivariate model. This makes sense as the treatment of MRSA infection requires higher trough-concentrations, thus being at higher risk of overexposure.

Pre-existing renal impairment or factors decreasing renal perfusion, such as heart failure and NSAIDs co-treatment, increase significantly the risk of vancomycin overexposure. This may be suggestive of insufficient vancomycin dosage adaptation in these cases. Through the univariate analysis, age is associated with a 20% risk increase for every ten years period. Vancomycin overexposure is more frequent in the presence of renal impairment. This reflects as well in vancomycin clearance, which is diminished in the overexposed group of patients (2.9 mL/min *vs* 4.3 mL/min). Mean daily dose and total

cumulative dose were not found to be risk factors for overexposure, suggesting that dosage adaptation is eventually made when trough-concentrations are rising. Another point of interest is renal function variables, especially the estimated creatinine clearance with Cockcroft-Gault equation, mean and maximal creatinine levels, outshining associated nephrotoxicity association with overexposure when included in the multivariate model. This can be related to the vicious circle described in the introduction.

With this vicious circle effect, it is difficult to determine the ultimate origin of the reciprocal causal relationship between induced-nephrotoxicity and overexposure. While cross-correlations tend to show some antecedence of vancomycin on creatinine changes, formal Granger tests suggest non-significant trends for reciprocal causation between vancomycin overexposure and occurrence of nephrotoxicity ($p=0.08$ both ways). This suggests that vancomycin overexposure might have some precedence in triggering renal impairment, instead of the opposite. As daily doses of vancomycin are positively correlated with future trough-concentrations but negatively with the past ones, this probably reflects here again a dosage adaption by prescribers in response to high trough-concentrations. The doses of vancomycin are themselves negatively correlated with creatinine levels both in the past and in the future, reflecting the dosage adaptation based on the renal function. Altogether, our exploration of chronological relationships between the development of vancomycin overexposure and of nephrotoxicity is consistent with the view that dosage adjustments for renal impairment are actually done by the prescribers, but possibly often with some delay, thus allowing vancomycin accumulation up to levels associated with nephrotoxicity. The vicious cycle can then progress, still encouraged by some delays in the readjustment of vancomycin dosages.

This study has several limitations. First, the number of patients included was limited, with a possible failure to statistically identify some significant trends despite real significance. The number of patients composing our reviewed population was determined by extrapolation, a margin of error is still existing regarding the model coefficients and the odds ratios that we obtained. Our study could have suffered from a selection bias, as we recruited only patients with TDM surveillance during vancomycin therapy, meaning they could have been suspected to be more likely subjects to nephrotoxicity by the medical team in charge. However, the utilization of TDM to monitor vancomycin treatment is widespread in our hospital, and it is very unlikely that a significant number of patients receiving vancomycin could escape the study because they had no TDM. The exclusion of patients matching the **C1'** criterion but failing to match the ultimate **C1 criteria** may be questionable regarding the representativeness of the sample. The follow-up of vancomycin trough-concentrations and creatinine in medical records was quite variable from a patient to another and information regarding population characteristics was sometimes unclear. We consider that the exclusion of these patients of uncertain status was the best approach, although it can have unduly cleaned up our results to some extent. Finally, our results possibly deserve

further modelling using modern population pharmacokinetic/pharmacodynamic approaches. This analysis was out of the scope of this study, but could lead to the elaboration of prediction tools possibly able to foresee the “trajectory” of vancomycin exposure and renal function in a given patient, so that corrective actions on dosage can be decided earlier and treatment safety is improved.

In conclusion, this study confirms a clear and marked association between vancomycin-associated nephrotoxicity and vancomycin overexposure. Medications or comorbid conditions favouring renal impairment, either by direct nephrotoxicity or by causing pre-renal dysfunction, are important risk factors to be taken into account by prescribers. This emphasizes the importance of thorough dosage adaptation during vancomycin therapy, with the aid of TDM and creatinine follow-up.

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