Vincristine-induced peripheral neuropathy in children with cancer

van de Velde, Mirjam Esther

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new insights on an old problem

Mirjam Esther van de Velde

COLOPHON

Vincristine-induced peripheral neuropathy in children with cancer – new insights on an old problem
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"I wish the Ring had never come to me.
I wish none of this had happened"

"So do all who live to see such times, but that is not for them to decide.
All we have to decide is what to do with the time that is given to us"

J.R.R. Tolkien – The Lord of the Rings

Dit proefschrift is opgedragen aan mijn lieve papa Hans Martin van de Velde

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Introduction

CHILDHOOD CANCER AND VINCRISTINE

Childhood cancer in high-income countries

Cancer during childhood is a rare disease. The global age-standardized incidence rate is only 16.2 per 100.000 person years [1]. The majority (82.2%) of the global burden of childhood cancer is situated in low, low-middle and middle income countries. However, childhood cancer is the leading cause of disease-related mortality during childhood in high-income countries[2,3]. Annually, over 400.000 children are diagnosed with cancer worldwide[4]. In Figure 1 the most common types of childhood cancer are depicted according to their contribution in disability-adjusted life years (DALY), which is a measure of disease burden that allows for cross-disease and cross-geography comparisons[1]. In the Netherlands, 576 children were diagnosed with any type of cancer in 2019[5]. These types of diagnoses are displayed in Figure 2 and show a similar distribution of cancer types as depicted in Figure 1.

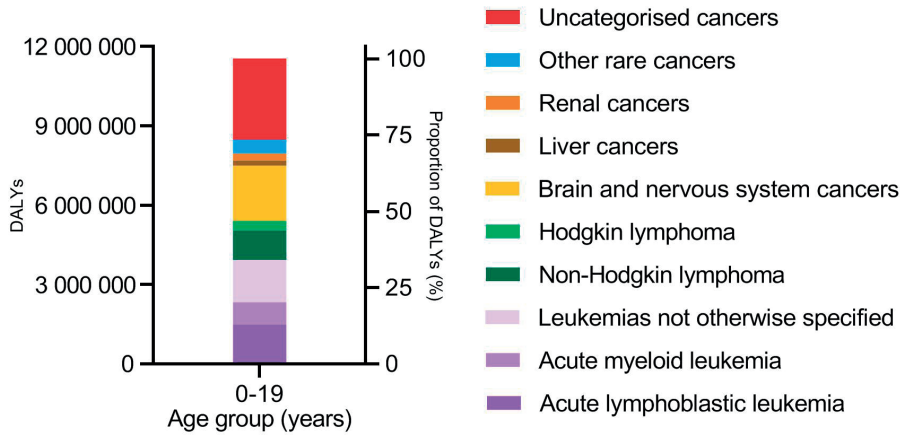

Figure 1. Absolute and proportional global DALY burden of childhood cancer types in 2017 of children aged 0-19 years

Source: Collaborators of the Global Burden of Disease Childhood Cancer[1]. DALY=Disability- Adjusted Life Years

The distribution of cancer types is different in children than in adults. In adults worldwide, the commonest cancer types are, in descending order of occurrence, airway (tracheal, bronchus and lung) cancer, colorectal-, breast-, non-melanoma skin-, prostate-, stomach-, liver- and cervical cancer, leukemia, and non-Hodgkin lymphoma[6]. These are mainly solid tumors and carcinomas, which are rare in childhood[1]. The majority of children with cancer who live in high-income countries have good outcomes, with approximately 80% of children surviving at least 5 years after their diagnosis[7,8]. These relatively high survival rates in childhood cancer make it possible to shift the focus of

research towards the optimization of cancer treatment and minimizing adverse side effects, instead of solely improving survival of cancer, although the latter, evidently, still is important.

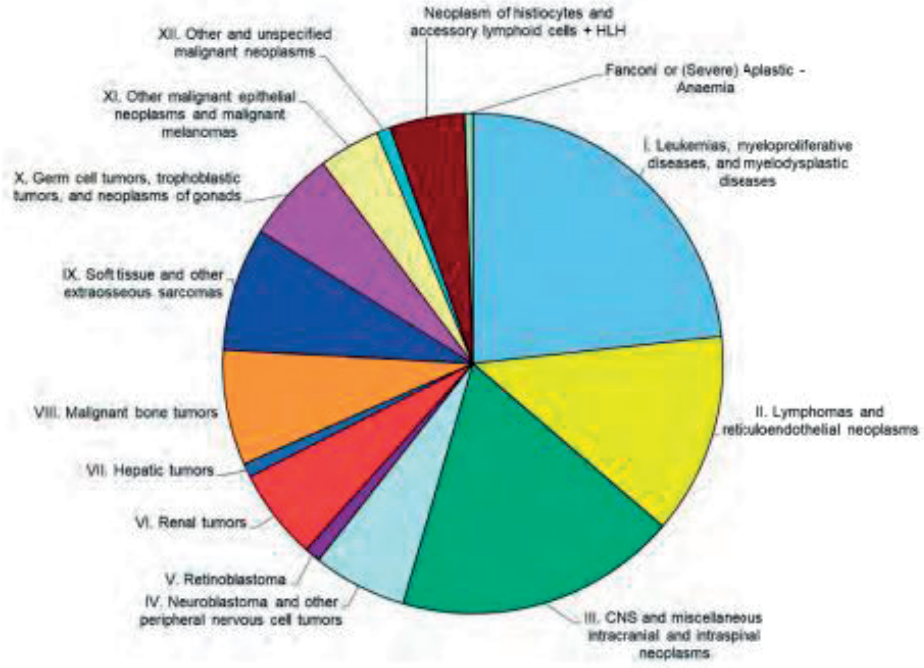

Figure 2: Distribution of pediatric cancer diagnosis in the Netherlands
Source: SKION Jaarverslag 2020 [5]. CNS: central nervous system, HLH: hemophagocytic lymphohistiocytosis

Chemotherapeutic treatment with vincristine: historical overview and working mechanism

Vincristine (VCR) is one of the oldest chemotherapeutic agents, used both in children and in adults for treating cancer. It is a naturally occurring alkaloid, that is present in very small quantities in the leaves of the periwinkle plant *Catharanthus roseus* (L.) G. Don of the family Apocynaceae. This plant is endemic in Madagascar. Currently, VCR is still extracted from the leaves of this plant[9,10]. The medicinal properties of VCR date back to the 17th century. Since then, extracts of the plant have been studied to stop hemorrhage and scurvy, as an analgesic in toothache, to heal wounds and diabetic ulcers, and to lower blood sugar levels[11-14]. However, its potential use as anticancer agent started after the observation by Noble et al. in 1957 that certain fractions of *Catharanthus roseus* resulted in a peripheral granulocytopenia and bone marrow sup-

pression[15]. Following this, Svoboda discovered in 1958 that certain extracts of this plant had antileukemic activity against the P-1534 lymphoblastic leukemia in DBA/2 mice[16]. The findings of this study led to more research into the anticancer properties of *Catharanthus roseus* momentum. In the following years, it was discovered that VCR was one of the few clinical valuable extracts of this plant[17]. Other, similar extracts were able to prolong life, but only VCR was able to cure P-1534 leukemia in mice with an acceptable range of side effects[18]. This led to the publication on the first successful clinical use of VCR, which was called leurocristine at the time, in 1962[19-25]. Since then, the use of VCR has been subject of numerous clinical studies and its applicability was shown many times since then.

The molecular structure of VCR is asymmetrical and dimeric. It composes of a dihydroindole nucleus, vindoline, linked by a carbon-carbon bond to an indole nucleus, catharanthine (Figure 3). VCR sulphate is 1:1 sulphate salt of the alkaloid extracted from *Catharanthus roseus.* The empirical formula of VCR sulphate is $C_{46}H_{56}N_4O_{10}H_2SO_4$ and the molecular weight is 923.1 kDa[26-28]. Currently, VCR is used more frequently and more effectively for treatment of pediatric cancer than for adult cancer. This is due to the fact that in general, pediatric cancers are more sensitive to VCR. Furthermore, children have a better tolerability to higher doses of VCR than adults[27,29,30].

In the 1960s and early 1970s the clinical application of VCR in pediatric oncology has been studied in many types of pediatric cancer, such as acute lymphoblastic leukemia, acute non-lymphoblastic leukemia, non-Hodgkin's lymphoma, Hodgkin's lymphoma, nephroblastoma, rhabdomyosarcoma, Ewing sarcoma, neuroblastoma, brain tumors (such as low-grade glioma and medulloblastoma), and retinoblastoma. It was first used as single-agent therapy in the early 1960s in patients who were refractory to conventional therapy at the time, which consisted of steroids, methotrexate, mercaptopurine, and alkylating agents. In general, children treated with VCR often had a partial or complete remission, especially in children with hematological malignancies. Unfortunately, these remissions as a result of single agent VCR treatment were often incomplete and brief[29,31]. The response rates improved with the use of combination chemotherapy. Currently, VCR is often incorporated within a multi-agent and multimodality treatment protocol for many oncologic disease types in pediatrics, both in hematological and solid tumors including brain tumors[30].

The working mechanism of VCR is based on the binding on microtubules within the cell. It interferes with the formation of the mitotic spindle, leading to an inability of the cell to perform mitosis[11,32-40]. Microtubules are the structural elements of the cytoskeleton within the cell and play a role in cell shape and motility, intracellular

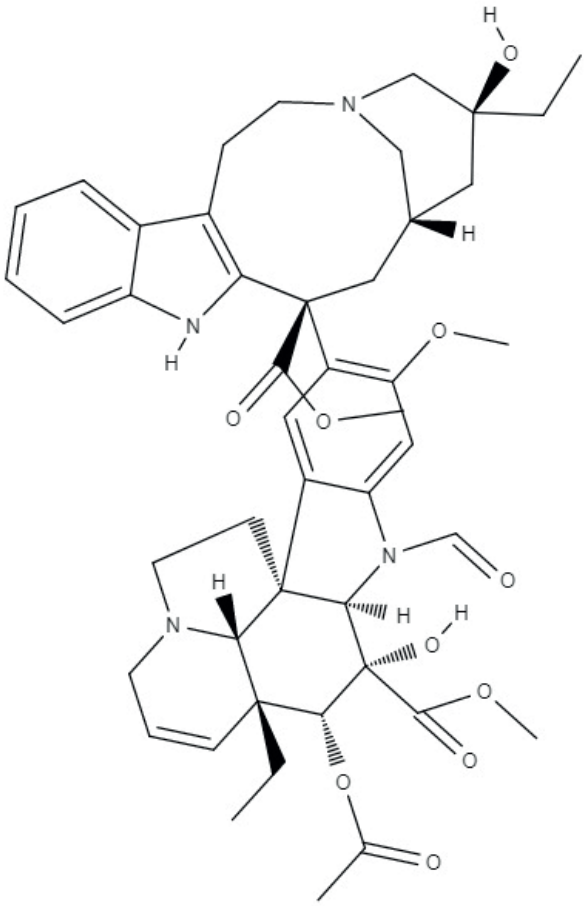

Figure 3. The two-dimensional structural formula of vincristine

transport, and secretion processes[41-43]. VCR works by binding to the tubulin part of microtubules (see Figure 4)[44]. Microtubules are composed of a primary skeleton structure of tubulin heterodimers (α- and β-tubulin subunits)[45]. During mitosis, microtubules grow and develop into the mitotic spindle, attaching with chromosomes. Depending on switching between either slow growth or rapid shrinkage, the microtubules either push or pull chromosomes towards the cell poles[46,47]. Figure 4 shows the binding of VCR to the β-subunit of the tubulin-dimer at the VCR binding site in the exterior of microtubules. VCR generally has two distinct binding sites on microtubules: a high affinity binding site at the microtubule plus end and a low affinity binding site on the tubulin along the microtubule surface[48]. Binding of VCR to the microtubules destabilizes microtubule formation, leading to depolymerization of the microtubules, which is the result of a longitudinal crosslink within the β-tubulin[49].

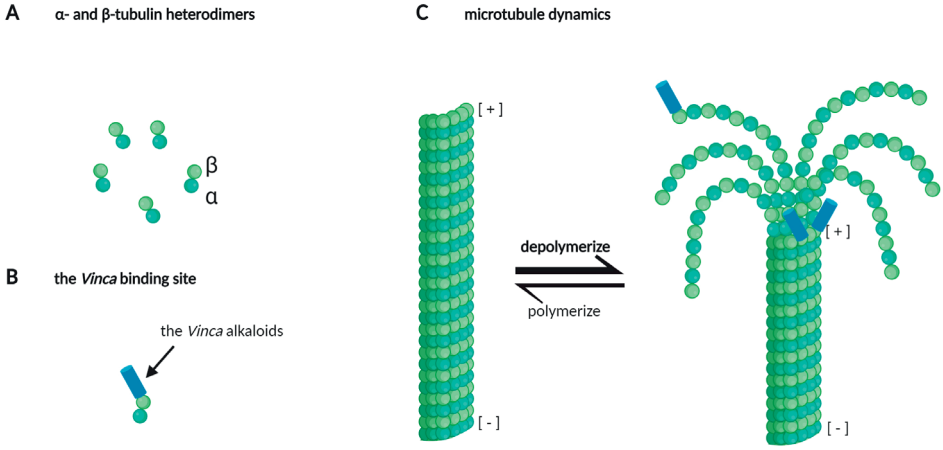

Figure 4. The structure of microtubule and effects of *Vinca* alkaloids on microtubule stability
A. Heterodimers of α- and β-tubulin assemble to form microtubules. B. The Vinca alkaloids bind to the β-subunit of tubulin dimer at the Vinca binding site in the exterior of microtubules. C. The Vinca alkaloids bind to the Vinca domain at the microtubule plus (+) ends which leads to depolymerization of microtubules. Figure created via biorender.com and adapted from Zhang et al.[44]

The mechanism of transportation of VCR into the cell is still not entirely elucidated, but in murine leukemia cells it was demonstrated that the transport mechanism depends on Michaelis-Menten kinetics, temperature, and competitive inhibition[50]. Furthermore, transport of VCR out of the cell depends on the adenosine triphosphate (ATP) binding cassette (ABC) transporter family, ABCB1, ABCC1, ABCC2 and ABCC3[51]. Finally, it has been demonstrated that increased levels of cholesterol and phospholipids in the cell membrane account for lower VCR accumulation in murine leukemia cells[52].

VINCRISTINE-INDUCED PERIPHERAL NEUROPATHY

Unfortunately, besides the anti-mitotic anticancer effect, VCR treatment can also lead to VCR-induced peripheral neuropathy (VIPN), which is the most common type of toxicity of VCR[30]. It can lead to symmetric sensory and motor neuropathy[53-55]. Symptoms of VIPN are muscle weakness, areflexia, neuropathic pain, sensory loss, and sometimes vocal cord paresis/paralysis[30]. Furthermore, it can cause an autonomic polyneuropathy, leading to symptoms such as orthostatic hypotension and constipation[56,57]. It is a dose-dependent toxicity, with symptoms that can already start after a few administrations[58]. It was shown that VIPN affects health-related quality of life (HR-QoL) in adults[59] and in survivors of childhood cancer[60]. Tay et al. [60] have found that in a study group 101 children >4 years after treatment for acute lymphoblastic leukemia (ALL),

children with VIPN had a significant lower score with respect to physical and social functioning, compared to children post-treatment for ALL but without VIPN. Therefore, children with VIPN are limited in their development, due to the prolonged effect of VIPN on HR-QoL.

The pathophysiology of VIPN is complex. Within the immune system, VCR leads to the activation of immune cells, which in turn results into the release of pro-inflammatory cytokines and activation of the innate and adaptive immune system and neuro-inflammation[61-66]. Within the peripheral tissues, VCR leads to integrin expression, resulting in neuro-inflammation in two ways: first, within the endothelial tissue there is a migration and activation of the C-X-3-C motif chemokine receptor, macrophages, and the release of TNFα and interleukins. Second, expression of C-X-C Motif Chemokine Ligand 12 in the dorsal root ganglia leads to the attraction and activation of C-X-C motif chemokine receptor 4 T-lymphocytes and monocytes also resulting in neuro-inflammation[65,67-73]. Furthermore, directly within the peripheral neurons VCR has an effect at multiple levels. Within the microtubules the main action mechanism of VCR is altered retrograde and anterograde transport, which leads to neuronal cell membrane remodeling and Wallerian degeneration, together with changes to cell shape and cell stability. This results in an altered function of voltage-gated ion channels of sodium (Na), potassium (K), calcium (Ca) and the transient receptor potential channel (TRP) and neuronal cell membrane remodeling. This in turn contributes to an altered excitability of the peripheral neurons, thereby causing peripheral neuropathy[65,74]. Within the myelin VCR leads to loss of peripheral sensory fibers. Finally, VCR also has an effect on the mitochondria within the cell, where it leads to an altered function of the respiratory chain and thereby of mitochondrial function. VCR stimulates the release of reactive oxygen species, altered glial cell function and activation of apoptotic pathways, which, all together, result in an altered function of the same voltage-gated ion channels (Na, K and Ca), TRP and neuronal cell membrane remodeling. This results in an altered excitability of the peripheral neurons[65,74,75].

Treatment options of VIPN are scarce and only symptomatic. Autonomic neuropathy leading to constipation can successfully be treated by using laxatives, which is often prescribed prophylactically when VCR is administered. Furthermore, neuropathic pain can be treated specifically with specific analgesics such as gabapentin or amitriptyline or with more general analgesics such as opioids. However, the effect of these treatment options are suboptimal and future research is needed to determine the best treatment strategy[76]. There are no adequate treatments for muscle weakness, numbness or paresthesia. Often, if these symptoms are persisting or even progressive,

the only treatment option is dose reduction or cessation of VCR treatment, but this likely results in suboptimal anti-cancer treatment[30,75].

Assessment of VIPN

There is a lack of specific and properly validated instruments for the assessment of VIPN. This has led to the use of a wide range of different instruments, making the comparability between studies regarding VIPN difficult. Non-invasive assessment of VIPN can be difficult as (young) children are often incapable of verbally expressing their complaints. Therefore, detection of symptoms relies largely on proxy-assessment. An assessment tool that is frequently used both in clinical practice and for research purposes is the Common Terminology Criteria for Adverse Events (CTCAE)[77]. This is an instrument for the assessment of all adverse events possibly associated with cancer treatment. Furthermore, a few instruments have been developed for the assessment of peripheral neuropathy specifically in children, such as the pediatric-modified Total Neuropathy Scale (ped-mTNS)[57] and the Total Neuropathy Scale Pediatric Version (TNS-PV)[78]. Both instruments consist of a questionnaire part as well as a physical examination part. Assessing VIPN through either the ped-mTNS or the TNS-PV leads to more children with VIPN being identified than through the CTCAE[79,80]. Because of their superiority over the use of CTCAE assessment, the use of TNS-PV or ped-mTNS was recommended for the assessment of VIPN in children aged ≥ 6 years[81]. However, both instruments still have some limitations. Both of them have not been properly assessed on their psychometric qualities: the reliability was not (optimally) studied and there is no standard recall period, amongst other limitations[81]. Finally, both instruments are not suitable for the assessment of VIPN in children aged ≤ 6 years. Sometimes, electrodiagnostic testing is used[54,82], which showed conduction delays and decreased amplitudes in several neurons[82-84]. However, since the use of these tools is painful and invasive, they are not suitable for routine assessment either clinically or for research purposes.

Factors contributing to vincristine-induced peripheral neuropathy

Administration- and pharmacokinetic-related factors

The standard dose of VCR currently used in clinical practice is 1.5 mg/m^2 with a maximum dose of 2.0 or 2.5 mg. Studies using higher doses have shown that this, in general, leads to intolerable VIPN in children[85]. However, studies that investigated the association between dose and VIPN using standard doses, are inconclusive and contradictory [57,76,82,84,86]. As such, the exact relation between VCR dose as used in daily clinical practice and VIPN in children remains to be established.

VCR is usually administered as a short (1-5 minutes), intravenous push administration. However, small-sized studies in children have shown that by using longer lasting infusions of up to 120 hours, higher cumulative doses of 4.0 mg/m^2 are generally well tolerated [87,88]. This could be due to lower peak plasma levels that are associated with longer lasting infusions, as lower peak plasma levels have been shown to be associated with less VIPN[89].

Ever since the first clinical use of VCR in the 1960s, the pharmacokinetics (PK) of VCR have extensively been studied. Initially, this was done using radio-immunoassay. However, this method is not very specific to assess VCR PK[90,91]. In 1991, a sensitive high-performance liquid chromatography (HPLC) assay was developed, which led to the reliable assessment of VCR PK in children[92]. This was followed by an assay using liquid chromatography-tandem mass spectrometry (LC-MS/MS), which further improved VCR PK analyses in 2009[93,94]. Several studies that investigated the PK of VCR in children[58,90,95-97] have shown that there is a large interpatient variability in VCR clearance[58,90,95,97]. Moreover, some studies found a relationship between age and several PK parameters of VCR[58,90,95], whereas others did not[96,97]. Finally, several studies reported on the association between VCR PK and VIPN[56,78,90,97-99]. One study found an association between the level of M1, the main metabolite of VCR, and VIPN[99] and one study showed an association between the VCR area under the concentration time curve (AUC) and VIPN[78], whereas the other studies did not find any association between PK and VIPN.

Genetic factors

The individual exposure to VCR with a standardized dose as well as the development of VIPN are influenced by genetic factors in several pathways. Theoretically, there are several mechanisms by which genetic factors can be associated with VIPN. First of all, genetic aberrations may influence the metabolism and PK of VCR. This could lead to an altered exposure of VCR in the central and peripheral compartment, and thus to either a higher or lower risk of VIPN. Secondly, genetic factors could alter the sensitivity of neurons to VCR, thereby increasing or decreasing the risk of developing VIPN. A well-known example of this is the motor and sensory neuropathic disease Charcot-Marie-Tooth (CMT). This is the most common hereditary neuromuscular disorder. At least 25 genes are associated with the development of CMT[100]. Children with this disease can develop very severe VIPN, already after the first administration of VCR[101-120]. This is due to the fact that the peripheral nervous system of these patients is already damaged due to CMT and therefore very vulnerable to the damaging effect of VCR.

Another factor that alters the susceptibility of developing VIPN, is ethnicity. Caucasian children have a higher risk of developing VIPN than African-American children[121]. However, the risk of VIPN among children of other ethnicities is not well studied. Although to date, the rationale behind ethnicity-associated VIPN is not very well understood, it is most likely related to genetic differences in children with different ethnicities.

The ethnic difference in the development of VIPN is probably in part explained by genetic differences in subtypes of the cytochrome P450 (CYP) 3A family of enzymes. This family of enzymes is important for the metabolism of VCR into M1 in the liver. This can be done by CYP3A4 and CYP3A5, although the latter has an up to 14 times higher intrinsic clearance in vitro than the first[122]. There is an unequal racial distribution in humans between CYP3A4 and CYP3A5. In African-Americans, the occurrence of CYP3A5 is present in 80% of people, whereas in Caucasians CYP3A4 is predominant in 80% of people. Therefore, people with CYP3A5 metabolize VCR quicker than people with CYP3A4, leading to lower exposure to VCR, and most often to less VIPN. However, although the relation between CYP3A subtype and the development of VIPN has been frequently studied, results remain inconclusive[86,98,99,123-125].

Besides CYP3A, various studies on pharmacogenomics of VCR have identified single nucleotide polymorphisms (SNPs) and genetic pathways that seem to be associated with VIPN. These factors are summarized in Table 1. However, to confirm their potential role in the development of VIPN, future studies are needed to validate these findings.

Concurrent azole treatment

As previously mentioned, VCR is hepatically metabolized using the CYP450 family of enzymes. Therefore, drugs that have the potential to increase VCR plasma levels are those with either an inhibiting effect of CYP450 or those that inhibit the P-glycoprotein-mediated VCR efflux[126]. The group of medication in which this mechanism is most frequently studied is the azole antifungals. These drugs are used for the prevention and treatment of (systemic) fungal infections. These fungal infections are common in intensely treated pediatric oncology patients, such as children with leukemia. The increased levels of VCR caused by the concurrent use of azole antifungals and VCR can lead to more (severe) VIPN. Research has shown that, although results were sometimes contradictory, concurrent use of VCR and azole antifungals leads to an increase in the incidence and severity of VIPN[127-132].

Table 1. Summary of study results regarding associations between SNP's and VIPN

SNP/genetic pathway studied	Studies demonstrating a lower risk on VIPN	Studies demonstrating a higher risk on VIPN	Studies not demonstrating an association with VIPN
CYP3A4		Aplenc[124], Egbelakin[99] and Kishi[123]	Guilhaumou[86], Moore[98], Ceppi[125]
VDR intron 8 pathway		Kishi[123]	
Glucocorticoid pathway		Kishi[123]	
Phase II pathway			Kishi[123]
Antimetabolite pathway			Kishi[123]
MDR1			Plasschaert[56]
ABCB1	Ceppi[125]		Guilhaumou[86]
ACTG			Ceppi[125]
ACTG1		Ceppi[125]	
CAPG*	Ceppi[125]	Ceppi[125]	
MAP4			Ceppi[125]
TUBB1			Ceppi[125]

**Different SNPs are associated with either an increased or a decreased risk of VIPN, SNP: single nucleotide polymorphism, VIPN: vincristine-induced peripheral neuropathy, CYP: cytochrome P-450, VDR: Vitamin D Receptor, miR: microRNA*

OUTLINE OF THIS THESIS

Despite the fact that VCR is already being used for decades, VIPN is still a major concern in pediatric oncology, with dose reductions or even cessation of VCR treatment sometimes being the only solution to reduce VIPN. Since VCR is a key component of many pediatric oncology treatment protocols all over the world, optimization of VCR treatment is of high importance. The goal of studies on optimization of VCR treatment is to maximize efficacy while minimizing (unnecessary) toxicity. Therefore, it is important to study VCR and VIPN using all currently available analytic tools. The availability of a sensitive LC-MS/MS assay to study PK parameters of VCR as well as the more widespread use of genome wide high-throughput genetic tests to study pharmacogenomics of VCR are valuable tools that can be used in our endeavors to optimize VCR treatment. These techniques should be used to study the effect of contributing factors to VIPN, as summarized in this introduction: ethnicity, dose, PK and administration duration. Moreover, an important issue to take into account when studying VIPN is the limited comparability of results of different studies making it difficult to draw robust conclusions on the influence of certain factors on VIPN. To this end, it is important to create more unity in the tools that are used to assess VIPN in children. Finally, although it is known that VIPN is a serious toxicity, it is currently

unknown what the effect of VCR is on daily functioning and health related quality of life (HR-QoL) of children. This is important to assess, since it helps to identify specific aspects of VIPN that have a high impact on HR-QoL. In this thesis, the above-described aspects of VIPN are studied, all with the aim to optimize VCR treatment for children with cancer.

In **Chapter 2** the relevant literature on contributing factors on VIPN is summarized by means of a systematic review. In **Chapter 3** the development and validation of the Dutch version of the ped-mTNS, a tool that can be used to assess VIPN in children, is described. **Chapter 4** reports on the effect of VIPN on health-related quality of life during treatment of cancer in children. Furthermore, **Chapter 5** presents the results of a randomized clinical trial that studied the effect of administration duration of VCR (push injections versus one-hour infusions) on the development of VIPN in children. Furthermore, This is followed by a study of the PK of VCR in children and its relation to VIPN and administration duration of VCR in **Chapter 6**. Then comes **Chapter 7**, which describes the results of a study regarding the pharmacogenomics of VCR in children. Finally, in **Chapter 8** we describe a study protocol investigating therapeutic drug monitoring of VCR in a specific population. All these studies are put in perspective, also with a look into the future of VCR and VIPN research in the general discussion in **Chapter 9**.

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Vincristine-induced peripheral neuropathy in children with cancer: a systematic review

Mirjam E. van de Velde, Gertjan L. Kaspers, Floor C.H. Abbink, Abraham J. Wilhelm, Johannes C.F. Ket, Marleen H. van den Berg

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ABSTRACT

Vincristine-induced peripheral neuropathy (VIPN) is a dose-limiting side effect of vincristine (VCR) treatment in children, leading to diminished quality of life. Much remains unknown about the underlying mechanisms of VIPN. This review systematically summarizes the available literature concerning contributing factors of VIPN development in children. Studied factors include patient characteristics, VCR dose, administration method, pharmacokinetics, and genetic factors. Furthermore, this review reports on currently available tools to assess VIPN in children. In total, twenty-eight publications were included. Results indicate that Caucasian race, higher VCR dose, older age and low clearance negatively influence VIPN, although results regarding the latter two factors were rather conflicting. Moreover, genetic pathways influencing VIPN were identified. Furthermore, the studied tools to assess VIPN seriously impairs comparability across study results. Studying the factors and their interactions that seem to influence VIPN in children, should aid in personalized VCR treatment, thereby increasing VCR effectiveness while minimizing toxicity.

INTRODUCTION

Since the introduction of vincristine (VCR) in 1962 has it been used as a chemotherapeutic agent in the treatment of various types of both adult and pediatric cancers. VCR is a vinca alkaloid derived from the plant *Catharanthus roseus* [1]. It causes restriction of tumor growth through its interference with the microtubules in the mitotic spindle [2,3]. The main side effect of VCR is neurotoxicity, a dose-limiting side effect causing peripheral and mostly symmetric sensory-motor neuropathy [4-6]. Other side effects of VCR include syndrome of inappropriate antidiuretic hormone secretion [7-10], myelosuppression [11,12] and alopecia [11,12]. Clinical symptoms of VCR-induced peripheral neuropathy (VIPN) include muscle weakness, areflexia, neuropathic pain and sensory loss, amongst others. Furthermore, it can cause autonomic polyneuropathy resulting in symptoms such as orthostatic hypotension and constipation.

In VIPN-affected patients the longer neurons such as the more distal neurons in the (lower) limbs are mainly affected [13-17].The symptoms of VIPN often develop already after a few administrations of VCR and in most cases symptoms disappear a few months after discontinuation of VCR therapy [18]. However, some children experience long-term sequelae, clinically established by symptoms such as permanent loss of deep tendon reflexes (DTR) and decreased motor functions [5,19].

In order to thoroughly study the influence of several factors on VIPN, it is important to use valid and reliable assessment tools.

In clinical practice the diagnosis of VIPN is hard to establish due to its heterogenic clinical presentation. Moreover, for young children it is difficult to describe their complaints, which is information necessary to accurately diagnose VIPN. The National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) are frequently used for assessing the degree of VIPN. This tool assesses the severity of several types of adverse events [20]. However, it has been demonstrated that the CTCAE has floor- and ceiling effects when it comes to assessing VIPN [21]. As a consequence, other methods to assess VIPN have been studied [21-24]. Currently, no golden standard is available to assess VIPN and most of the tools used have limited value in young children, making it difficult to accurately quantify VIPN in this group of patients [22]. Another method used to diagnose VIPN as well as to elucidate the possible pathophysiology of peripheral nerve damage is electrodiagnostic testing [25,26]. This method, however, is more invasive and painful which makes it not suitable for routine assessment of VIPN in children.

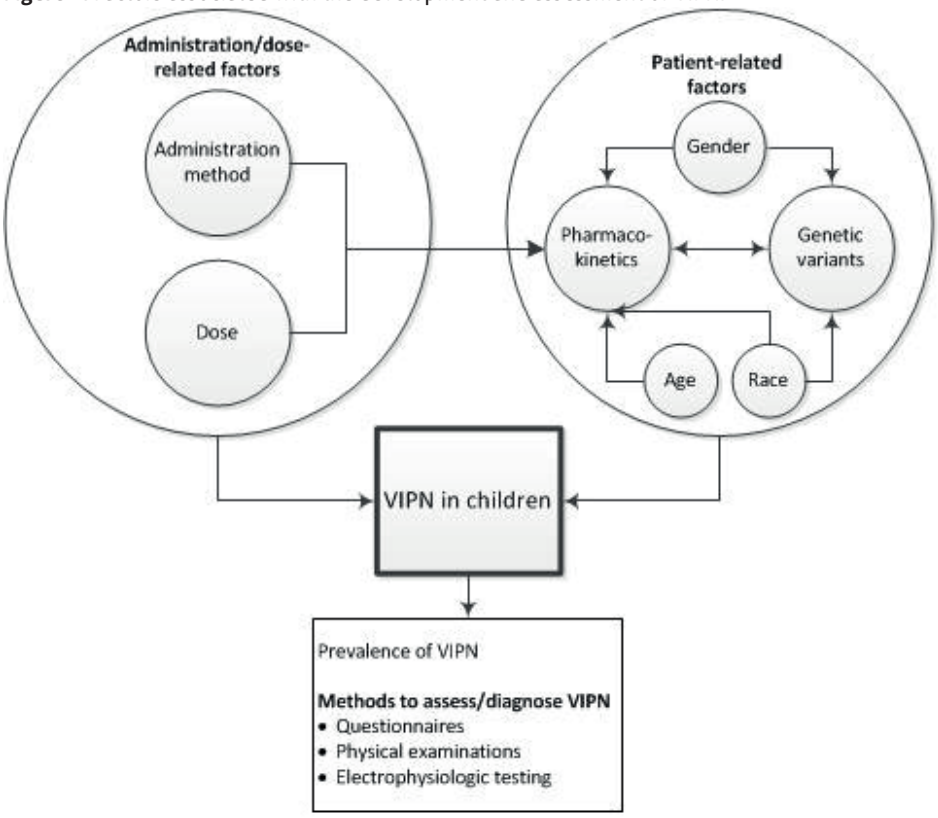
The mechanisms underlying VIPN have been studied frequently. However, most studies included adults only. As a consequence, there is a gap of knowledge about the various factors that influence VIPN in children. Moreover, results of previous studies regarding the relation between VIPN and age seem to be contradictory [4,5,27-30]. Also the exact role of VCR pharmacokinetics (PK) on VIPN in children remains to be established[31,32].

The development and severity of VIPN in children is determined by multiple factors which often are inter-related (see Figure 1). One of these factors is the VCR dose administered. Standard dosing in children nowadays is 1.5-2.0 mg/m^2 with a maximum of 2.0-2.5 mg, and in infants the dose is 0.05-0.065 mg/kg. Furthermore, in general, VCR is administered with a minimum interval of one week [33]. Larger doses or smaller time intervals may result in unacceptable toxicity [28]. However, this toxicity can also be the result of interactions with other drugs. The most frequently studied interaction is that of VCR and azole antifungals. Multiple publications showed increased VIPN after concomitant azole therapy [34-36]. Furthermore, the method of administration seems to affect VIPN development and severity [37]. In clinical practice, VCR is administered intravenously through bolus injections or prolonged infusions. Gidding et al. have shown that VCR bolus injections induce high peak-plasma concentrations of VCR, which in turn seems to be related to the development of VIPN in children [38]. In addition, continuous infusion of VCR seems to increase the systemic exposure of VCR without significantly increasing the development of VIPN [39]. However, strong evidence from high-quality studies is lacking.

Another factor influencing the risk of developing VIPN concerns the patients' PK profile [31]. Since the early nineties, high performance liquid chromatography (HPLC) with sensitive detection limits has become the standard method for most of the PK studies due to which accurate data on PK measures of VCR have become available [33]. Although these data have demonstrated large inter-individual variability in children [40], studies indicate that in general VCR plasma clearance is higher in children than in adults [41,42]. Since high clearance of VCR is associated with diminished drug exposure [39], this may lower the risk of developing VIPN. However, genetic factors also influence this risk, either through influencing the PK of VCR or through genetically increased susceptibility of developing VIPN [28,40]. Several abnormalities in DNA such as single nucleotide polymorphisms (SNPs) with different pathways have been studied in VIPN-affected children [28,31,43]. Genetic factors influence the development of VIPN by altering the clearance on one hand and by influencing the patients' susceptibility for developing VIPN on the other hand [28].

All in all, many factors influence the development of VIPN in children. However, results of previous studies investigating the exact impact of each of these factors on VIPN as well as the relation between these factors and VIPN are inconclusive, making it difficult to unravel the rather complex mechanism(s) underlying VIPN in children. This review aims to systematically summarize the available evidence concerning the various factors that contribute to the development of VIPN in children. Factors that are being studied include VCR dose, administration method, PK and genetic factors. Furthermore, this review reports on the psychometric qualities of the current available tools to assess and diagnose VIPN in children.

Figure 1. Factors associated with the development and assessment of VIPN.



VIPN: Vincristine Induced Peripheral Neuropathy

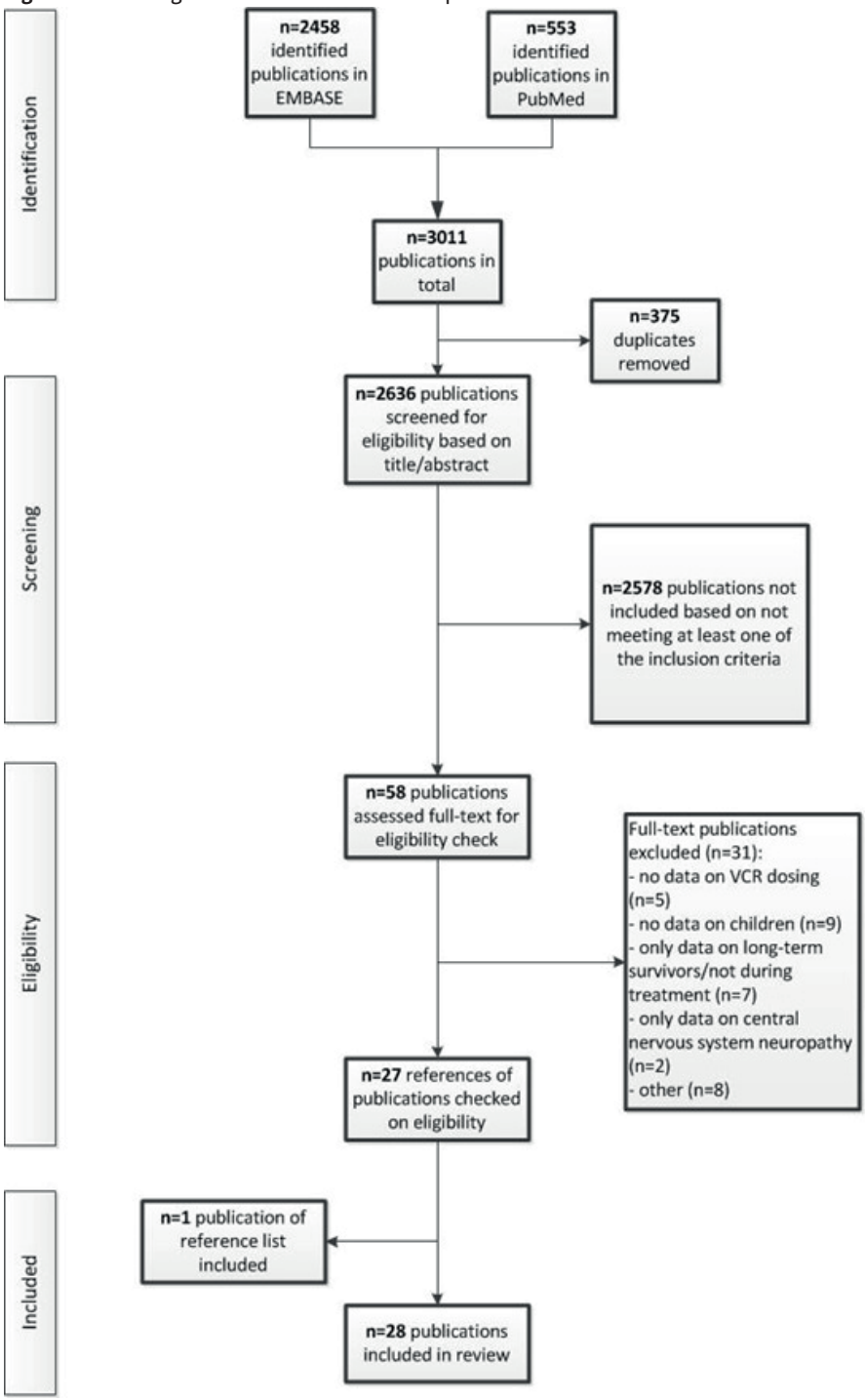
METHODS

A review protocol was developed based on the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA)-statement (www.prisma-statement.org). Subsequently, a literature search was performed using MEDLINE/PubMed (from 1945 up to December 13th 2016) and EMBASE/Ovid (from 1980 up to December 13th 2016). The following key words were used: "pediatrics", "vincristine" and "neurotoxicity". The exact search queries for the literature searches in the several databases are stated in Supplementary table 1.

Publications were deemed eligible for inclusion in case they met the following inclusion criteria: (a) study population: study should include a pediatric oncology population consisting of at least five children, (b) treatment protocol: children should have received multiple VCR administrations, (c) study measurements: study measurements should have been performed on patients who still receive regular VCR administrations for their underlying oncologic disease, (d) data on VCR dose should have been reported, (e) study outcomes: data on peripheral neuropathy should have been reported, (f) publications should have been written in English. Letters to the Editor, case-reports, historic reviews and (conference) abstracts were excluded.

After removing any duplicates, two reviewers (MV and MB) independently selected publications meeting the inclusion criteria based on title, abstract or both. Discrepancies between reviewers were resolved by consensus. Subsequently, all selected publications were obtained in full-text in order to extract data using a standardized data extraction form as well as a quality assessment form. In the data extraction form data were collected on study characteristics of participants, methods and results on VIPN prevalence, method of assessment, dose and administration method, genetic factors, and other factors. The quality assessment form was an adaptation of the Quality Assessment Tool for Quantitative Studies [44] and was used to systematically assess publication quality and risk of bias. This form included 10 questions on the methodological quality of the study. Based on the answers to these questions a total Quality Assessment Score was calculated, which was used to categorize the publication as low-, moderate- or high-quality studies. Reference lists of relevant publications were screened for possible additional inclusions. Results were reported according to the factors described in Figure 1.

Figure 2. Flow diagram of search and selection process



RESULTS

The search yielded a total of 3011 publications (Figure 2). After removing 375 duplicates, 2636 publications were screened for eligibility based on title and/or abstract. This resulted in the exclusion of 2578 publications, as these did not meet inclusion criteria. The remaining 58 publications were then screened for eligibility based on full-text versions. Finally, a total of 27 publications met the inclusion criteria and were included in the current review. The hand-search resulted in one additional publication. Characteristics regarding study design and methods are depicted in Table 1, whereas in Table 2 the actual results of the included studies are described.

Prevalence rates of VIPN among children treated with multiple VCR administrations were reported in 25 of the 28 included studies and appeared to vary greatly. In the studies that used CTCAE criteria to evaluate VIPN and in which VIPN was defined as grade 2-4, prevalence varied between 12% and 38% [17,27,28,32,42,45-47]. Kishi et al. [48] defined grade 3 and 4 of CTCAE as VIPN and found a prevalence of 13%. In other studies in which VIPN was defined as either having loss of DTR or having CTCAE grade I or higher, prevalence varied from 20% to 100% [29,31,32,39,42,49]. In studies using other tools to assess VIPN prevalence rates appeared to vary as well: 30-55% (World Health Organization (WHO) neurotoxicity criteria [50,51]), 55-87% (pediatric-modified Total Neuropathy Scores (ped-mTNS) [22,24]), 12-95% (clinical neurological assessment of VIPN symptoms [52,53]), 18-82% (CTCAE constipation criterion [43]), and 72-79% (electrophysiological methods [25,53,54]). Furthermore, Lavoie Smith et al. [29] combined different tools to diagnose VIPN and did not report a prevalence, but found an incidence of 78% when using the Total Neuropathy Score – Pediatric Vincristine (TNS-PV), 69-74% when using CTCAE criteria, and 44% when using the so-called FACES pain scale.

Table 1: Study design, population characteristics, administered vincristine dose, and methods used to assess vincristine-induced peripheral neuropathy in the included studies

Author (year of publication)	Design & Method							
	Study design	Study population characteristics					VCR dose (single) administered	Method used to assess VIPN
		Number of participants	Disease studied	Age at study(years)	Gender	Race/ethnicity		
Anghelescu et al. (2011) [17]	Cohort	153	ALL	1-5: 47.1% 6-10: 27.5% 11-15: 16.2% 16-20: 9%	Male: 56% Female: 44%	White: 68% Black: 16% Hispanic: 12% Asian or other: 4%	Treated according St. Jude Total XV protocol: 1.5 – 2.0 mg/m^2, cumulative dose: 61-63 mg/m^2	CTCAE 2.0 item pain. Additional pain assessment by FLACC, FACES or NPS
Aplenc et al. (2003) [55]	Case control	533	ALL	Genotyped cohort: <2: 16%, 2-5: 54%, >5: 30% Controls cohort: <2: 16%, 2-5: 56%, >5: 28%	Genotyped & controls cohort: Male: 32% Female: 68%	Genotyped cohort: African American: 6%, other: 94%; Control cohort: African Americans: 4%, other: 96%	Treated according to CCAG-1891 protocol: 1.5 mg/m^2, cumulative dose: 46.5-64.5 mg/m^2	CCG toxicity criteria
Arzanian et al. (2009) [56]	Cohort	75	Nephroblastoma (n=5) and ALL (n=70)	0-2: 9%, 2-4: 21%, 4-6: 19%, 6-8: 20%, 8-10: 11%, 10-12: 11%, 12-14: 9%	Male: 54% Female: 46%		1.5 mg/m^2, maximum 2 mg, cumulative dose: 6 mg	Neurologic examination
Ceppi et al. (2015) [45]	Cohort	275	ALL	<10: 80%, >10: 20%	Male: 55.3%, Female: 44.7%	Caucasian: 100%	Treated according DFCI 87-01, 91-01, 95-01 or 00-01 protocol: 1.5-2 mg/m^2, cumulative 73.5-74 mg/m^2	CTCAE 3.0 items sensory, motor and autonomic neuropathy

Table 1: Study design, population characteristics, administered vincristine dose, and methods used to assess vincristine-induced peripheral neuropathy in the included studies (*continued*)

Author (year of publication)	Design & Method							
	Study design	Study population characteristics					VCR dose (single) administered	Method used to assess VIPN
		Number of participants	Disease studied	Age at study(years)	Gender	Race/ethnicity		
Courtemanche et al. (2015) [26]	Cohort	17	ALL, lymphoma and solid tumor	Mean (range): 11.2 (1.5-17)	Male: 35% Female: 65%		1.5 mg/m^2, cumulative dose: 3.5-17.61 mg/m^2	Nerve conduction studies, neurologic examination
Crom et al. (1994) [42]	Cohort	54	ALL	Mean: 4.3, range: 0.16-18	Male: 40.7% Female: 59.3%	White: 87% Black: 13%	Treated according to St. Jude Total Therapy Study XI or XIII: 1.5 mg/m^2, maximum 2 mg. Cumulative dose: 51 mg/m^2. Infants: 0.05 mg/kg	NCI CTC on 13 categories
Diouf et al. (2015) [28]	Cohort	321	ALL	<1: 2.5%, 1-10: 59.8%, >10: 37.7%	Male: 58.3% Female: 41.7%	European: 65.1% African: 13.4% Asian: 0.6% Hispanic: 13.7% Other: 7.2%	Treated according to St. Jude Total XIIIB protocol: 1.5 mg/m^2, maximum 2 mg, cumulative dose: 54 mg/m^2. Treated according to protocol COG AALL0433: 1.5-2 mg/m^2, maximum 2-2.5 mg, cumulative dose: 78-97.5 mg/m^2	CTCAE 1.0 and modified Balis scale for neuropathy (based on CTCAE 2.0)
Egbelakin et al. (2011) [31]	Cohort	107	ALL			Caucasian: 93.4% Hispanic/Latino: 5.6% African American: 0.01% Asian: 0.01%	Treated according to COG AALL0331, AALL0232, AALL0434, and AALL01P protocol: 1.5 mg/m^2, maximum 2 mg	CTCAE 3.0 items sensory, motor and autonomic neuropathy

Table 1: Study design, population characteristics, administered vincristine dose, and methods used to assess vincristine-induced peripheral neuropathy in the included studies (*continued*)

Author (year of publication)	Design & Method							
	Study design	Study population characteristics					VCR dose (single) administered	Method used to assess VIPN
		Number of participants	Disease studied	Age at study(years)	Gender	Race/ethnicity		
Gilchrist et al. (2009) [24]	Cross-sectional	20	ALL, lymphoma and solid tumors	Mean (SD): 10.6 (4.7), range: 5-18	Male: 70% Female: 30%	White non-Hispanic: 90%, Hispanic: 5% Asian: 5%	Cumulative (SD): 21.1 (20.6) mg/m^2, range 3.2 – 46.5 mg/m^2	ped-mTNS
Gilchrist et al. (2013) [22]	Cross-sectional	82	ALL, lymphoma and solid tumors	Mean (SD) cases: 9.56 (3.1), mean (SD) controls: 9.61 (3.1) , total range: 5-18	Male: 36.6% Female: 63.4%		Cumulative: 16.6 mg/m^2, range 3.0-28.5 mg/m^2	ped-mTNS and BOT-2
Gilchrist et al. (2014) [21]	Cross-sectional	83	ALL, lymphoma and solid tumors	Mean (SD): 10.7 (3.9), range: 5-18	Male: 40% Female: 60%		Cumulative (SD): 15.9 (8.0) mg/m^2, range 1.8-39.0 mg/m^2	ped-mTNS and CTCAE 3.0
Guilhaumou et al. (2011) [46]	Cohort	26	Solid tumors	Mean (SD): 8.8 (4.2), range: 2-16		Caucasian	1.5 mg/m^2, maximum 2 mg, cumulative: mean (SD) 7.35 (5.3) mg/m^2	CTCAE 3.0 items pain, peripheral neurotoxicity and gastrointestinal toxicity
Gutierrez et al. (2015) [47]	Cohort	142	ALL	<1: 0.7%, 1-10: 87.9%, >10: 11.3%, mean 5.1	Male: 57% Female: 43%		Treated according to LAL/SHOP 94/99/2005 protocol: 1.5 mg/m^2, cumulative: 15-30 mg/m^2	CTCAE 1.0

Table 1: Study design, population characteristics, administered vincristine dose, and methods used to assess vincristine-induced peripheral neuropathy in the included studies (*continued*)

Author (year of publication)	Design & Method							
	Study design	Study population characteristics					VCR dose (single) administered	Method used to assess VIPN
		Number of participants	Disease studied	Age at study(years)	Gender	Race/ethnicity		
Kellie et al. (2004) [39]	Cohort	16	CNS tumors	Mean: 6.5, range: 1.7-15.8	Male: 69% Female: 31%		1.5 mg/m^2 in 24 hours followed by 96 hours continuous infusion of 0.5 mg/m^2/24 hours. Repeated after 21 days. Total dose (depending on BSA): 2 x 3.5-4 mg/m^2	Modified CTCAE
Kishi et al. (2007) [48]	Cohort	240	ALL	<1: 4% 1-10: 65.2% >10:30.8%	Male: 58.3% Female: 41.7%	White: 70% Black: 18% Asian: 12% Other: 0.4%	Treated according to St. Jude Total XIIIB protocol: 1.5 mg/m^2, maximum 2 mg, cumulative dose: 54 mg/m^2. Treated according to protocol COG AALL0433: 1.5-2 mg/m^2, maximum 2-2.5 mg, cumulative dose: 78-97.5 mg/m^2	CTCAE 1.0 items constipation, sensory and motor neuropathy
Lavoie - Smith et al. (2013) [23]	Cohort	65	ALL	Mean (SD): 6.38 (4.35), range: 2-19	Male: 52.3% Female: 47.7%	White: 88% African American: 8% Not reported: 5%	Treated according to COG protocols AALL0331, AALL0232, AALL07P4, AALL0932, no or other protocol: 1.5 mg/m^2, maximum: 2 mg, cumulative (SD): 12.6 (4.9)	TNS-PV, Balis scale and FACES pain scale

Table 1: Study design, population characteristics, administered vincristine dose, and methods used to assess vincristine-induced peripheral neuropathy in the included studies (*continued*)

Author (year of publication)	Design & Method							
	Study design	Study population characteristics					VCR dose (single) administered	Method used to assess VIPN
		Number of participants	Disease studied	Age at study(years)	Gender	Race/ethnicity		
Lavoie - Smith et al. (2015) [29]	Cohort	109	ALL	Mean (SD): 7.45 (4.10), range: 1-19	Male: 47.2% Female: 52.8%	Caucasian: 88% African American: 5.8% Asian: 0.3% Unknown: 6.0%	Treated according to COG protocols AALL0232, AALL0331: 1.5 mg/m^2, maximum: 2 mg	TNS, CTCAE 4.0, TNS-PV, Balis, PNPS and FACES pain scale
Lombardi et al. (2015) [27]	Cohort	45	Hepatoblastoma	Mean: 1.33, range: 0.17-12			Treated according to Study INT-0098 and COG AHEP031 protocol: 1.5 mg/m^2 for children >10kg and 0.05 mg/kg/dose in children <10kg, total doses: 4-24	CTCAE 4.0
Lopez-Lopez et al. (2016) [51]	Cohort	152	ALL	Mean (SD): 5.49 (3.4)	Male: 56.6% Female: 43.4%		Treated according to LAL-SHOP 94/99/2005 protocol: induction: 4x1.5 mg/m^2, later phases of treatment: 6-16x1.5mg/m^2	WHO criteria
Moore et al. (2011) [32]	Cohort	50	Pediatric oncology	Mean: 6.5, range: 1.0-16.25	Male to female: 1:1	Caucasian: 86% Hispanic: 4% Asian: 4% Pacific Islander: 4% Australian Aboriginal: 2%	Mean (SD) VCR dose: 1.48 (0.25) mg/m^2, range: 0.67-2.0	CTCAE 3.0

Table 1: Study design, population characteristics, administered vincristine dose, and methods used to assess vincristine-induced peripheral neuropathy in the included studies (*continued*)

Author (year of publication)	Design & Method							
	Study design	Study population characteristics					VCR dose (single) administered	Method used to assess VIPN
		Number of participants	Disease studied	Age at study(years)	Gender	Race/ethnicity		
Pinkerton et al. (1988) [52]	Cohort	5	Neuroblastoma and sarcoma	Mean: 10.4, range: 2-25			1.5 mg/m^2 followed by 0.5 mg/m^2/day, cumulative dose of 3.5 mg/m^2	Clinical examination
Plasschaert et al. (2004) [43]	Cohort	52	ALL	1: 8%, 2-5: 48%, 6-9: 17%, 10-16: 27%	Male: 62% Female: 38%	White: 98% Other: 2%	Treatment according to DCOG protocol ALL-9: 2 x 1.5 mg/m^2 (during measurements)	CTCAE item constipation
Reinders - Messelink et al. (2000) [50]	Cohort	17	ALL	Mean: 5.8, range: 4-12.6	Male: 65% Female: 35%		Treatment according to DCOG protocol ALL-8: 1.5 mg/m^2, maximum: 2-2.5 mg, maximum cumulative dose: 18 mg	CMAP, SNAP, vibration sense and modified WHO neurotoxicity score
Renbarger et al. (2008) [49]	Cohort	113	ALL	Caucasians: mean age: 4.95 (±3.08) African Americans: mean age: 8.2 (±4.8)	Male: 50% Female: 50%	Caucasian: 81% African American: 19%	Caucasians: mean cumulative dose (SD): 48.52 (14.52) mg/m^2, African Americans: mean cumulative dose (SD): 42.44 (11.62) mg/m^2	CTCAE 3.0
Toopchizadeh et al. (2009) [53]	Cohort	42	Non-CNS tumors	Mean: 6.2 mg/m^2, range: 3-11	Male: 55% Female: 45%		1.5 mg/m^2, mean cumulative dose in patients with mild VIPN (SD): 4.31 (2.29) mg/m^2, mean cumulative dose in patients with severe VIPN (SD): 5.64 (2.43) mg/m^2	CMAP, SNAP and clinical examination

Table 1: Study design, population characteristics, administered vincristine dose, and methods used to assess vincristine-induced peripheral neuropathy in the included studies (*continued*)

Author (year of publication)	Design & Method							
	Study design	Study population characteristics					VCR dose (single) administered	Method used to assess VIPN
		Number of participants	Disease studied	Age at study(years)	Gender	Race/ethnicity		
Vainionpaä et al. (1993) [5]	Cohort	40	ALL	Mean: 4.9, range: 0.6-15.3	Male: 38% Female: 62%		Treated according Nordic regimen or BFM-83 protocol: 1.5-2 mg/m^2, cumulative dose: 12-22 mg/m^2	Neurologic examination according to Touwen, digit span test, head circumference, intracranial pressure by papilledema
Vainionpaä et al. (1995) [25]	Cohort	38	ALL	Mean: 5.3, range: 1.4-15.3	Male: 37% Female: 63%		Treated according Nordic regimen or BFM-83 protocol: 1.5-2 mg/m^2, cumulative dose: 12 mg/m^2	SSEP, neurologic examination according to Touwen
Yildiz et al. (2016) [54]	Cohort	25	Pediatric oncology	Mean (SD): 7.2 (4.8), range: 1-16	Male: 64% Female: 36%		Mean cumulative dose (SD): 8.4 (4.3) mg/m^2	CMAP, SNAP and medical chart review

VCR: vincristine, VIPN: Vincristine-Induced Peripheral Neuropathy, ALL: Acute Lymphoblastic Leukemia, CTCAE: national cancer institute Common Toxicity Criteria for Adverse Events, FLACC: Face, Legs, Activity, Cry, Consolability scale, NPS: Neuropathic Pain Scale, CCG: Children's Cancer Group, DFCI: Dana-Farber Cancer Institute, NCI CTC: National Cancer Institute Common Toxicity Criteria, COG: Children's Oncology Group, SD: standard deviation, ped-mTNS: pediatric modified Total Neuropathy Score, BOT-2: Bruininks-Osteretsky Test of motor Proficiency version 2, CNS: Central Nervous System, BSA: Body Surface Area, TNS-PV: Total Neuropathy Score – Pediatric Version, TNS: Total Neuropathy Score, PNPS: Pediatric Neuropathic Pain Scale, WHO: World Health Organization , DCOG: Dutch Childhood Oncology Group, CMAP: Compound Muscle Action Potential, SNAP: Sensory Nerve Action Potential, BFM: Berlin-Frankfurt-Munster, SSEP: somatosensory evoked potentials.

Table 2: Results of studies included in this review on vincristine-induced peripheral neuropathy in children with cancer

Author (year of publication)	Results					Study Quality
	Prevalence of VIPN-induced symptoms	Factors associated with VIPN				
		Dose and administration method	PK measures	Genetic variants	Other factors	
Anghelescu et al. (2011) [17]	• Grade 2-4 VIPN: 34.9% • Recurrence of pain after initial VIPN episode: 16%	No significant relation between VIPN and cumulative dose (p=0.45) or dose preceding VIPN diagnosis (p=0.59).			No significant relation with gender (p=0.29) or age (p=0.51) and VIPN. Significant relation with race (p<0.01) with white race most affected.	High
Aplenc et al. (2003) [55]				Significant association between *CYP3A4*1B* & *CYP3A5*3* expressers versus non-expressers and VIPN (p=0.024, p=0.021). No significant results after correction for multiple comparisons.		High
Arzanian et al. (2009) [56]	• Decreased DTR Achilles: 78% • Decreased DTR patella: 53% • Muscle weakness: 70% • Constipation: 12%				No significant relation between gender and VIPN. Significant relation between age and VIPN, children <4 less VIPN than older children (p<0.05).	Low

Table 2: Results of studies included in this review on vincristine-induced peripheral neuropathy in children with cancer (*continued*)

Author (year of publication)	Results					Study Quality
	Prevalence of VIPN-induced symptoms	Factors associated with VIPN				
		Dose and administration method	PK measures	Genetic variants	Other factors	
Ceppi et al. (2015) [45]	• Low-grade VIPN: 20%, • High-grade VIPN: 10.6%			Significant association between SNPs in *ACTG1* (p=0.02), two in *CAPG* (p=0.04 and p=0.03) and *ABCB1* (p=0.01) and VIPN. SNPs in *CAPG* associated with lower risk of high grade VIPN (p=0.009) and SNPs in *ABCB1* associated with lower risk of low grade VIPN (p=0.02).	Significant relation between age and VIPN, more VIPN in older children (p<0.008). Significant relation between protocol and VIPN, less VIPN in more recent protocols (p=0.003). Significant relation between risk group and VIPN, more VIPN in high risk patients (p=0.01).	High
Courtemanche et al. (2015) [26]	• Neuropathic pain: 82% • Hypesthesia: 29% • Motor deficit: 76% • Walking difficulties: 76% • Loss of walking ability: 24% • Areflexia: 100% • Cranial nerve palsy: 12% • Constipation: 53%					Low

Table 2: Results of studies included in this review on vincristine-induced peripheral neuropathy in children with cancer (*continued*)

Author (year of publication)	Results					Study Quality
	Prevalence of VIPN-induced symptoms	Factors associated with VIPN				
		Dose and administration method	PK measures	Genetic variants	Other factors	
Crom et al. (1994) [42]	• Abdominal pain: 59% • Constipation: 33% • Motor dysfunction: 28%	No significant relation between single-course or cumulative AUC and VIPN.	Clearance (mL/min/kg): mean: 19.9 (range: 1.3-62.3) Clearance normalized to body weight <10 years: mean 20.4 versus >10 years: 9.4 ml/min/kg (p=0.024)		No significant relation between age and VIPN.	Low
Diouf et al. (2015) [28]	Prevalence grade 2-4 VIPN: 29% (St. Jude's Total XIIIB cohort), 22% (COG cohort)	Significant association between VCR dose and VIPN (p<0.0001)		Significant association between SNP in the *CEP72* gene and VIPN. Severity of VIPN higher in SNP *CEP72* population compared to other genotype (p<0.0001).	Significant relation between gender and VIPN, girls more affected than boys (p=0.02), Significant relation between age and VIPN, more VIPN in older children (p=0.03). Significant relation between race and VIPN, European race most severely affected (p=0.002).	High

Table 2: Results of studies included in this review on vincristine-induced peripheral neuropathy in children with cancer (*continued*)

Author (year of publication)	Results					Study Quality
	Prevalence of VIPN-induced symptoms	Factors associated with VIPN				
		Dose and administration method	PK measures	Genetic variants	Other factors	
Egbelakin et al. (2011) [31]	Experienced VIPN: 89-100%.		Significant relation between M1 produced by *CYP3A5* expressers and non-expressers (p=0.0004). Significant inverse relation between M1 and VIPN grade (p=0.03).	Significant association between *CYP3A5* expressers compared to non-expressers and VIPN (89% vs. 100%, p=0.03).		Moderate
Gilchrist et al. (2009) [24]	• Sensory symptoms: 60% • Motor symptoms: 55% • Impairments in pin sensibility: 30% • Impairments in vibration: 50% • Distal muscle strength: 90% • DTR: 95%					Moderate
Gilchrist et al. (2013) [22]	• Sensory VIPN: 39% • Motor VIPN: 78%.	No significant relation between cumulative VCR dose and VIPN.				Moderate

Table 2: Results of studies included in this review on vincristine-induced peripheral neuropathy in children with cancer (*continued*)

Author (year of publication)	Results					Study Quality
	Prevalence of VIPN-induced symptoms	Factors associated with VIPN				
		Dose and administration method	PK measures	Genetic variants	Other factors	
Guilhaumou et al. (2011) [46]	VIPN in 33% of patients.	Significant relation between VCR dose (p=0.006, regression coefficient =0.29) and VIPN, significant relation between BSA and VIPN (p=0.006) and age and VIPN (p=0.002).	No significant relation between PK measures and VIPN: clearance: p=0.12, $T_{1/2}$: p=0.11, AUC: p=0.84.	No significant association between *CYP3A4*, *CYP3A5* or *ABCB1* genotype and VIPN (p=1.0, p=0.72 and p=0.62).	Significant relation between VIPN and age (p=0.002, regression coefficient =0.34).	Moderate
Gutierrez et al. (2015) [47]	Induction: 25.4% VIPN, later treatment phase: 4.2% VIPN.			No significant association between SNP in *CEP72* gene and VIPN.	No significant relation between gender (p=0.34) or age (p=0.51) and VIPN.	Moderate
Kellie et al. (2004) [39]	CTCAE grade 1 or higher: 65% of all VCR courses.		Mean values of clearance: 203 mL/min/m^2, volume of distribution at steady state: 358 L/m^2, plasma concentration at steady state: 2.0 µg/L.			Moderate
Kishi et al. (2007) [48]	CTCAE grade 3 or 4: 13%.			Significant association between *CYP3A5*, *VDR* intron 8 and glucocorticoid group genotype and VIPN (p=0.006, p=0.04 and p=0.24, respectively).	No significant relation between age or gender and VIPN. Significant relation between race and VIPN, with white race more affected (p=0.0003).	High

Table 2: Results of studies included in this review on vincristine-induced peripheral neuropathy in children with cancer (*continued*)

Author (year of publication)	Results					Study Quality
	Prevalence of VIPN-induced symptoms	Factors associated with VIPN				
		Dose and administration method	PK measures	Genetic variants	Other factors	
Lavoie Smith et al. (2013) [23]		Significant relation between TNS-PV score and cumulative VCR dose (p=0.05, r=0.41).	Significant relation between AUC and VIPN (r=0.41, p=0.05).			Moderate
Lavoie Smith et al. (2015) [29]	78% VIPN with TNS-PV, 96% pain with FACES pain scale.				No significant relation between gender or race and VIPN. Significant relation between age and TNS-PV score (r=0.31, p<0.0001).	High
Lombardi et al. (2015) [27]	Grade 2 or 3 VIPN: 38%, pain requiring gabapentin: 29%, decreased mobility: 22%.				Age <24 months in all but 2 VIPN cases (p=0.13, OR: 2.88).	Low
Lopez-Lopez et al. (2016) [51]	No VIPN: 67.8% Low-grade VIPN: 18.4% High-grade VIPN: 11.8% No data: 2%	No significant relation between number of VCR doses received and VIPN.		Significant association between 12 SNPs in 3 genes and VIPN (5 SNPs in *ABCC2*, 6 SNPs in *ABCC1* and 1 SNP in *ABCB1*).	No significant relation between age, gender and VIPN.	Moderate

Table 2: Results of studies included in this review on vincristine-induced peripheral neuropathy in children with cancer (*continued*)

Author (year of publication)	Results					Study Quality
	Prevalence of VIPN-induced symptoms	Factors associated with VIPN				
		Dose and administration method	PK measures	Genetic variants	Other factors	
Moore et al. (2011) [32]	Grade 3-4 VIPN: 12%, grade 1-2 VIPN: 88%.		No significant relation between VCR clearance or AUC and VIPN. No significant relation between PK and age or gender.	No significant association between CYP genotype and VIPN.	No significant relation between race and VIPN.	High
Pinkerton et al. (1988) [52]	Leg pain: 33%, abdominal pain: 11%.		Mean clearance: 8.5 mL/min/kg, mean steady state concentration: 1.68 ng/mL, average time to reach steady state: 18.3 hour, median concentration at 1 hour: 6.7ng/mL.			Low
Plasschaert et al. (2004) [43]	Constipation grade 1-2: 50-82%, constipation grade 3-4: 18-47%.		No significant relation between clearance, AUC, $t_{1/2}$ and obstipation.	No significant association between SNPs in the *MDR1* gene or haplotype status and constipation (p>0.10 all genetic variants).		High

Table 2: Results of studies included in this review on vincristine-induced peripheral neuropathy in children with cancer (*continued*)

Author (year of publication)	Results					Study Quality
	Prevalence of VIPN-induced symptoms	Factors associated with VIPN				
		Dose and administration method	PK measures	Genetic variants	Other factors	
Reinders-Messelink et al. (2000) [50]	• CTCAE grade 1-2 VIPN: 55% • Grade 3-4 WHO toxicity: 0% • Mild sensory disturbance: 27% • Loss of Achilles DTR: 91% • VPT disturbance >2 SD: 22%.				Significant relation between gender and VIPN, with boys more affected than girls with lower action potentials on SNAP's ($p<0.01$). No significant relation between age and SNAP/CMAP or VPT.	Moderate
Renbarger et al. (2008) [49]	African Americans with VIPN: 4.8% Caucasians with VIPN: 34.8%.	Significant relation between VCR dose and VIPN, with 0.1% of African Americans needing dose reductions vs. 4% of Caucasians: ($p<0.0001$).			Significant association between race and VIPN, with African-Americans less affected than Caucasians ($p<0.0001$).	Moderate
Toopchizadeh et al. (2009) [53]	Electrophysiological evidence of VIPN: 79%.	No significant relation between dose and mild/moderate VIPN and severe VIPN ($p=0.236$).			Significant relation between gender and sural latency, with more prolongation in female patients (p value not reported). No significant relation between age and VIPN.	Low

Table 2: Results of studies included in this review on vincristine-induced peripheral neuropathy in children with cancer (*continued*)

Author (year of publication)	Results					Study Quality
	Prevalence of VIPN-induced symptoms	Factors associated with VIPN				
		Dose and administration method	PK measures	Genetic variants	Other factors	
Vainionpaä et al. (1993) [5]	• Depressed patellar DTR after induction: 71% • Depressed patellar DTR after CNS therapy: 79% • Depressed Achilles DTR after induction: 87% • Depressed Achilles DTR after CNS therapy: 87% • Gross motor disorder after induction: 23% • Gross motor disorder after CNS therapy: 33%. • Fine motor disorders after induction: 0% • Fine motor disorders after CNS: 5%	Significant relation between risk group and VIPN, with patients in SR group (total cumulative dose: 22mg/m^2) more affected than patients in IR/HR group (total cumulative VCR dose: 12 mg/m^2) ($p<0.02$).			Significant relation between inability to walk and age, with younger patients more affected than older ($p<0.03$).	Moderate
Vaionpaä et al. (1995) [25]		Significant relation between cumulative VCR dose of 12 mg/m^2 and VIPN ($p<0.001$).				High

Table 2: Results of studies included in this review on vincristine-induced peripheral neuropathy in children with cancer (*continued*)

Author (year of publication)	Results					Study Quality
	Prevalence of VIPN-induced symptoms	Factors associated with VIPN				
		Dose and administration method	PK measures	Genetic variants	Other factors	
Yildiz et al. (2016) [54]	• Sensory symptoms: 28% • Motor symptoms: 72% • Asymmetric symptoms: 24% • Walking difficulties: 60% • Absent DTR: 100% • Motor NCS abnormalities: 92% • Sensory and motor NCS abnormalities: 8%					Low

VIPN: Vincristine-Induced Peripheral Neuropathy, PK: pharmacokinetic, CYP: Cytochrome P 450, DTR: Deep Tendon Reflex, SNP: Single Nucleotide Polymorphism, AUC: Area Under the Curve, COG: Children's Oncology Group, BSA: Body Surface Area, CTCAE: Common Toxicity Criteria of Adverse Events, VCR: vincristine, TNS-PV: Total Neuropathy Score - Pediatric Version, WHO: World Health Organization, VPT: Vibration Perception Threshold, SD: standard deviation, SNAP: Sensory Nerve Action Potential, CMAP: Compound Muscle Action Potential, CNS: Central Nervous System, SR: Standard Risk, IR: Intermediate Risk, HR: High Risk, NCS: nerve conduction studies

Tools to assess or diagnose VIPN

In previous studies several tools have been used to measure or diagnose VIPN. These are summarized in Table 3. Sixteen of the 28 studies included in the current review used CTCAE criteria (items: peripheral sensory neuropathy, peripheral motor neuropathy, pain, several autonomic dysfunctions, and constipation [17,21,28,29,31,32,39,43,45,46,48]) for assessing VIPN in children. However, in five studies it was not mentioned which items were used [27,42,47,49,55]. The CTCAE shows great resemblance with the WHO neurotoxicity score used in two other studies [50,51]. Kellie et al. [39] used an adaptation of the CTCAE in order to develop a neuropathy grading system. Furthermore, Gilchrist et al. [21,22,24] described the development of the ped-mTNS, which is an adaptation of the Total Neuropathy Score (TNS) [57]. Psychometric properties of the ped-mTNS were evaluated in children aged 5-18 years with multiple, non-CNS tumor types. Internal consistency of the tool appeared to be sufficient and inter-rater and test-retest reliability were acceptable. Compared to the CTCAE criteria, ped-mTNS identified 40% more subjects with sensory neuropathy and 15% more subjects with motor neuropathy. Except for strength testing, no significant correlation between ped-mTNS scores and combined motor and sensory CTCAE scores were found.

Lavoie-Smith et al. [23] also developed a VIPN assessment tool which was based on the TNS: the TNS-PV. This tool showed good internal consistency . The TNS-PV was compared with three other methods to assess VIPN: the CTCAE, the Balis grading scale and the FACES pain scale. TNS-PV scores were significantly correlated with CTCAE scores (p=0.01) and with Balis scores (p=0.01). Furthermore, the TNS-PV and CTCAE appeared to be sensitive to change over time. VIPN could validly be assessed through the TNS-PV in 95% of all children aged 6 and older. The CTCAE and Balis tools showed significant floor effects and the construct validity of the Balis tool seemed inferior compared to the TNS-PV. The FACES Pain Scale was valid, sensitive, responsive and feasible for assessing VIPN-related pain severity in children of all ages. Moreover, TNS-PV assessments were more sensitive in detecting VIPN than CTCAE scores [29].

Electrodiagnostic testing was used in five of the included studies. Besides assessing the degree of VIPN, electrodiagnostic testing can give insight into the pathophysiology of VIPN. Vainionpaä et al. [25] used somatosensory evoked potentials (SSEPs) at the right median and right posterior tibial nerve in a group of 38 children with ALL. Conduction delays were found in the tibial (p=0.001 for standard risk patients; p=0.0014 for intermediate and high risk patients) and median nerve (p=0.001 for standard risk patients; p=0.049 for intermediate and high risk patients) after VCR therapy. In addition, Reinders- Messelink et al. [50] used surface electrodes to determine the conduction velocity of the median, ulnar, peroneal and sural nerves as well as the sensory

nerve action potentials (SNAPs) and compound muscle action potentials (CMAP) in 17 children with ALL. Results indicated that amplitudes of action potential of peroneal and sensory ulnar and median nerves decreased during VCR treatment. The CMAPs of the upper extremity nerves and the sural SNAP appeared not to be significantly associated with cumulative VCR dose. Furthermore, no effect of VCR treatment was found on conduction velocities.

Toopchizadeh et al. [53] performed sensory and motor nerve conduction studies on the median and ulnar nerves of children receiving VCR treatment. Furthermore, sensory nerve conduction was performed on the sural nerves and motor conduction studies on the tibial and peroneal nerves as well. Significant negative correlations were found between cumulative VCR dose and the amplitude of tibial (p=0.007), peroneal (p=0.013), median (p=0,003) and ulnar (p=0.006) CMAPs, while significant positive correlations were found between cumulative VCR dose and sensory latencies of ulnar (p=0.027) and median (p=0.05) nerves.

Table 3: Tools used for VIPN assessment

Tools used for VIPN assessment	Description of tools
Balis grading scale	Tool to grade neuropathy in children based on a 4-point scale.
BOT-2	Tool to age specifically assess motor proficiency in patients with mild to moderate functional deficits.
NCI CTCAE	Toxicity criteria for adverse events, such as peripheral sensory- and motor neuropathy, constipation and neuralgia. Grading scale of 0-5, 0 meaning no toxicity and 5 meaning death.
FACES pain scale	Tool measuring pain in children (aged 3-8 years) by face drawings depicting increasing degrees of distress due to pain.
Ped-mTNS	Adaptation of TNS consisting of a questionnaire combined with standardized physical examination. It tests sensory symptoms, pain, motor function, autonomic function, light touch sensation, vibration sensation, pin sensation, distal strength, and DTR in children (aged 5-18).
PNPS	Five-point scale for grading pain in children.
TNS-PV	Adaptation of TNS consisting of a questionnaire combined with standardized physical examination; more abbreviated than ped-mTNS. It tests pain, temperature sensibility, vibration sensation, strength, DTR, autonomic function, and hoarseness/vocal cord function in children (aged 6-18).
WHO criteria	Grading scale providing great similarity to NCI CTCAE assessment
Nerve conduction studies	Electrophysiological assessment of conduction velocity and amplitude. It comprises SSEP, SNAP and CMAP amongst others.

VIPN: Vincristine-Induced Peripheral Neuropathy, BOT-2: Bruininks-Osteretsky Test of motor Proficiency version, CCG: Children's Cancer Group, NCI CTCAE: National Cancer Institute Common Toxicity, SSEP: somatosensory evoked potentials, SNAP: sensory nerve action potential, CMAP: compound muscle action potential, ped-mTNS: pediatric modified Total Neuropathy Score , TNS: Total Neuropathy Score, DTR: deep tendon reflexes, PNPS: Pediatric Neuropathic Pain Scale , TNS-PV: Total Neuropathy Score – Pediatric Version, WHO: World Health Organization

Finally, Yildiz et al. [54] performed sensory and motor nerve conduction studies on median, ulnar, tibial, peroneal and sural nerves in children with several types of malignancies. This showed significant differences between normal group data and patients on motor abnormalities, with decreased distal CMAP and prolonged distal negative peak duration ($p<0.01$). All other sensory and motor modalities were not significantly different between patients and controls.

Patient-related factors

Age

Contradictory results regarding the relation between VIPN and age at study have been reported. Two studies report a significantly higher prevalence of VIPN in younger children [5,27], whereas five other studies report a significantly higher prevalence in older children [28,29,45,46,56]. However, the two studies reporting a higher prevalence in younger children had a low [27] or moderate [5] quality score, while two of the five [28,45] studies reporting a significantly higher prevalence in older children were rated as high-quality studies. Moreover, seven studies did not find an association between age and VIPN in children [17,42,47,50,51,53,54].

In two studies, both rated as high-quality, in which the relation between clearance of VCR and age was investigated, no effect of age on clearance was identified [32,43]. In 12 publications the effect of age on VIPN was not studied.

Gender

Three studies found a significant relation between gender and VIPN [28,50,53], with two of them indicating that girls were more affected by VIPN [28,53], whereas the other study found boys to be at higher risk [50]. In addition, seven studies did not find a significant relation between gender and VIPN in children [17,29,47-49,54,56], of which three were rated as high-quality. In 18 publications the effect of gender on VIPN was not studied.

Race

Information on race and/or ethnicity of the included children was reported in 14 studies [17,23,27-29,31,42,43,46,48,55], with a five of them actually investigating the relation between race and VIPN. In four studies a significant relation was demonstrated [17,28,48,49], all showing that children of Caucasian origin are at higher risk of developing VIPN than children of African-American or other origin. One study did not find a significant relation between race and VIPN, but race was unequally distributed with 88% Caucasian patients [29].

Genetic variants

Studies focusing on the relation between genetic variants and VIPN have identified several correlations. A summary of these associations is outlined in Table 4. The SNPs most frequently studied were the CYP3A family of enzymes. Results of these studies have provided inconclusive results. Of the seven studies that investigated the role of CYP3A enzymes and VIPN in children, three found a significant correlation between these factors [31,48,55], whereas four studies did not [32,45,46,51]. Aplenc et al., [55], Egbelakin et al. [31] and Kishi et al. [48] all found that children with active subtypes of CYP3A5 had a significantly decreased risk of developing VIPN in comparison with children who did not express an active form of CYP3A5 (these children were either expressing an inactive form of CYP3A5 or an active form of CYP3A4). Furthermore, Kishi et al. [48] demonstrated that SNPs in the vitamin D receptor (*VDR)* intron 8 polymorphism and glucocorticoid-related genes (CYP3A, p-glycoprotein (*MDR1*), glucocorticoid receptor (*NR3C1*) and VDR) were associated with VIPN (p=0.04 and p=0.024, respectively). However, Guilhaumou et al. [46], Moore et al. [32] and Ceppi et al. [45] found no relation between the incidence of VIPN and the CYP3A family of enzymes. However, in the case of Moore et al. [32] this is most likely due to the small number of patients with different CYP variants. Besides the CYP3A family of enzymes, Guilhaumou et al. [46] studied *ABCB1* and found no significant relation with the incidence of VIPN (p=0.62). Besides *CYP3A4/5* subtypes, other genetic pathways were studied as well. Plasschaert et al. [43] studied several SNPs in the *MDR1* gene. This gene codes for a P-glycoprotein and may play a role in the metabolism of VCR. There were no associations between VIPN, measured as severity of constipation, and SNPs in the *MDR1* gene. Moreover, no significant correlations between *MDR1* SNPs and PK measurements were found.

Ceppi et al. [45] studied genetic subtypes of the *CYP3A5* enzymes as well as genetic subtypes in the *ABCB1* genes and in genes coding for tubulin- and actin-associated proteins (*TUBB1, MAP4, ACTG, ACTG1* and *CAPG*). They found an inverse association with one SNP in the *ABCB1* gene that was associated with lower risk of VIPN (p=0.02),and one SNP of the *CAPG* gene that was associated with lower risk of VIPN (p=0.009), both in univariate and multivariate analysis. Contradictory, another SNP in the *CAPG* gene as well as another SNP in the *ACTG1* gene appeared to be associated with higher grades of VIPN (p=0.02 and p=0.008, respectively).

Diouf et al. [28] performed a genome-wide association study in children with ALL to identify SNPs correlated with VIPN. The only SNP they identified as having a relation with VIPN was in the *CEP72* gene (p<0.0001). This gene codes for a centrosomal protein that is involved in microtubule formation. Severity of VIPN was higher in the population with a SNP in the promoter region coding for this protein than in other genotypes (56 % in SNP of *CEP72* group vs. 21% in other group). Contradictory to

the results of Diouf et al., Gutierrez et al. [47] found no significant association between VIPN and the previously mentioned SNP in the *CEP72* gene in a Spanish cohort of 142 children with ALL. However, the study of Diouf et al. had a high quality score, whereas the study of Gutierrez et al. had a moderate score.

Lopez-Lopez et al. [51] studied eight candidate genes for its relation with VIPN. These genes were vincristine transporters. They found 12 SNPs with a significant association with VIPN, all in the vincristine transporter genes (5 in *ABCC2* (p values between p=0.0004 and p=0.049), six *ABCC1* (p values between p=0.008 and p=0.047) and one in *ABCB1* (p=0.021)). After correction for multiple analysis, only two SNPs in *ABCC2* remained significantly associated with VIPN (p=0.036 in both SNPs after correction). Of the studies investigating genetic factors, four corrected for multiple comparisons [43,48,51,55], whereas six did not.

Table 4: Summary of study results regarding associations between SNP's and VIPN

SNP/genetic pathway studied	Studies finding a lower risk on VIPN	Studies finding a higher risk on VIPN	Studies not finding an association with VIPN
CYP3A4		Aplenc[55], Egbelakin[31] and Kishi [48]	Guilhaumou [46], Moore [32], Ceppi [45] and Lopez-Lopez [51]
CYP3A5	Aplenc[55], Egbelakin[31] and Kishi [48]		Guilhaumou [46], Moore [32], Ceppi [45] and Lopez-Lopez [51]
VDR intron 8 pathway		Kishi [48]	
Glucocorticoid pathway		Kishi [48]	
Phase II pathway			Kishi [48]
Antimetabolite pathway			Kishi [48]
MDR1			Plasschaert [43]
ABCB1	Ceppi [45]		Lopez-Lopez [51], Guilhaumou [46]
ACTG			Ceppi [45]
ACTG1		Ceppi [45]	
CAPG	Ceppi [45]	Ceppi [45]	
MAP4			Ceppi [45]
TUBB1			Ceppi [45]
CEP72		Diouf [28]	Gutierrez [47]
ABCC1			Lopez-Lopez [51]
ABCC2		Lopez-Lopez [51]	
ABCC3			Lopez-Lopez [51]
ABCC10			Lopez-Lopez [51]
RALBP1			Lopez-Lopez [51]

SNP: single nucleotide polymorphisms, VIPN: vincristine-induced peripheral neuropathy, CYP: cytochrome P-450, VDR: Vitamin D Receptor

Pharmacokinetics

Of the derived pharmacokinetic parameters from Moore et al. [32], based on VCR concentrations on five different time points the following was found: distribution volume (Vd, mean): 526 l/m^2, clearance (Cl, median): 482 mL/min/m^2, Area Under the Curve (AUC, median): 49.7 µg*h/L and halflife ($t_{1/2}$,mean): 12.3 h. No significant relation between these PK measures and severity of VIPN was established. Comparable results were found in the study of Plasschaert et al. [43] in which no significant relation between severity of VIPN (defined as severity of constipation) and VCR clearance, AUC, or $t_{1/2}$ was found. Both studies were rated as high-quality studies. Finally, Crom et al. [42] also could not establish the relation between VIPN grade and AUC, although most PK evaluations were performed after four or fewer courses of VCR, therefore the effect of cumulative dose of VCR on VIPN was limited. Clearance in this study was comparable to Moore et al. [32]: 482.4 mL/min/m^2.

In the study of Guilhaumou et al. [46], in which liquid chromatography-mass spectrometry was used to analyze intracellular VCR levels in mononuclear cells in nine blood samples over three courses in three schedules, no significant relation between VIPN incidence and PK parameters (Cl,$t_{1/2}$ and AUC) was found.

Egbelakin et al. [31] studied the relation between PK measures and genetic variants (*CYP3A5* subtypes vs. non *CYP3A5* subtypes). Sampling time points and method of PK analysis were not reported. They found a significant relation between the level of the main metabolite of VCR M1 and VIPN grade. In the study of Lavoie Smith et al. [23] blood samples were taken just prior to VCR administration andafter 5, 15 and 30 min. Moreover, in four of 65 children samples were drawn after 12 and 72 h. Methods used for PK analyses were not reported. In this study a significant correlation between higher VIPN grades and AUC was demonstrated.

VCR dose and administration-related factors

The single VCR doses used in the several studies varied. Mostly, a single dose of 1.5 mg/m^2 with a maximum of 2.0 or 2.5 mg once a week was administered [5,17,23,25-29,31,42,43,45-48,50,51,53,55,56], but also doses of 2.0 mg/m^2 with a maximum of 2.0 or 2.5 mg per week were used [5,17,25,28,45]. In two studies in which infants under 10 kg were studied, doses of 0.05 mg/kg/dose were used [27,42]. The cumulative doses applied in the included studies appeared to differ greatly, depending on the disease studied, the treatment protocol, and the risk stratification of the patient. In children with ALL cumulative doses ranged from 12.0 to 74.0 mg/m^2 [5,17,23,25,28,42,47-51,55]. In studies which included other types of pediatric malignancies the cumulative doses ranged from 4.0 to 16.6 mg/m^2 [21,22,24,26,46,53,54,56].

In the study of Kellie et al. [39], in which 16 children with a CNS tumor received two courses of 1.5-2.0 mg/m^2 of VCR through a bolus injection followed by a continuous infusion of 0.5 mg/m^2/day for four consecutive days with a cumulative dose of 7.5 mg/m^2, an increase in systemic exposure was obtained without an increase in VIPN severity. Pinkerton et al. (1988) [52] studied a regimen of five days continuous VCR infusion in which a cumulative dose of 4.0 mg/m^2 was administered in nine children. Results showed that a more than twofold dose escalation could be obtained without increasing the number of patients with VIPN.

In general, the studies investigating the effect of dose on the development or severity of VIPN provided inconclusive results. Six studies found an increased prevalence or severity of VIPN in relation with higher (cumulative) doses [5,23,25,28,46,53], whereas three other studies could not establish this relation [17,22,29]. However, the opposite was never observed.

DISCUSSION

Vincristine is among the most widely used chemotherapeutic agents for treatment of children with cancer. Unfortunately, VIPN is a frequently encountered side effect, not seldom leading to sub-optimal dosing from a therapeutic point of view [49]. Moreover, quality of life of VIPN-affected children is deprived , even after completion of VCR treatment [58]. To the best of our knowledge this is the first systematic review that studied all facets of VIPN, i.e. patient-associated factors, dose- and administration-related factors, pharmacokinetics, genetic factors, and tools to assess VIPN in children. The results of our review identified several possible predisposing factors of VIPN in children. First, there seems to be a relation with age, with older children being more likely to be affected than the younger ones. In addition, race most likely influences VIPN, with Caucasian children being more affected than children of other races. Furthermore, results show that the reported incidence and prevalence of VIPN in children vary greatly, probably caused by the variety of tools used to assess VIPN. In addition, it has been demonstrated that the dose administered (cumulative as well as single dose), PK variables and several genetic pathways seem to influence VIPN.

However, it should be noted that many studies in which the associations between these influencing factors and VIPN were investigated, showed contradictory results. One of the factors that showed large discrepancies among several studies was age. From a theoretical point of view it can be reasoned that mostly infants/younger children should be at the highest risk of developing VIPN, since in younger children the development of their neural system and myelination of nerves is still incomplete [59]. Therefore, the largely unmyelinated peripheral nerves could be more susceptible to damage, such as VIPN, than older myelinated nerves. This hypothesis is supported by the fact that children with a demyelinating form of Charcot-Marie-Tooth disease are known to be more susceptible for VIPN [26,60-63]. On the other hand, PK variables are also influenced by age [64], although the precise impact of age on VCR metabolism is not yet fully elucidated. In two of our included studies no relation was found between age and several PK measures [32,43], whereas in other studies it was demonstrated that children metabolize VCR quicker than adults [41,42]. All in all, the question remains whether younger children are equal or faster VCR metabolizers than adolescents. In case (certain groups of) children indeed appear to metabolize VCR quicker, dose adaptation in these children might prevent VCR to reach toxic plasma levels, which in turn could lead to less VIPN. In any case, the exact impact of both age and VCR metabolism on VIPN in children should be further studied, as well as the relation between these two factors.

Besides age, race is also an important factor known to influence VCR metabolism. Race affects the distribution of CYP enzymes [31,49], which appeared to be associated with VIPN development in four studies. However, the role of race in VIPN is not yet fully understood, as in all studies investigating the effect of race on VIPN in children, children of Caucasian origin outnumbered children of other ethnicities. To determine the influence of race on VIPN development, studies must be undertaken in cohorts with a more diverse racial distribution among the several groups.

Moreover, race can affect PK parameters which in turn influences the risk of developing VIPN. However, these parameters also influence the therapeutic effectiveness of VCR [65]. Studies aimed at investigating the exact relation of race on VIPN as well as therapeutic effectiveness are highly advocated, as this new knowledge can help in developing optimal, personalized VCR dosing regimens.

Another factor of which the precise influence on VIPN needs to be clarified in order to make personalized VCR dosing possible, is the genetic pathways involved in VIPN. Results of this review show that variability in VIPN or VCR PK parameters is based on multiple genetic pathways. In general, there seem to be two genetic pathways through which VIPN development is influenced: by altering the susceptibility for VIPN development and/or by affecting VCR metabolism. Diouf et al. have shown the *CEP72* gene to be associated with VIPN development in children with ALL [28], whereas Stock et al. found the same association in adults with ALL [66]. The CYP pathway has shown to be associated with VCR metabolism, thereby influencing plasma levels of VCR [31,55]. However, the effect of several genetic factors on VIPN should be studied further. For example, it is likely that children who develop severe VIPN after a single dose of VCR are different than children developing VIPN after a higher cumulative dose of VCR, but genetic pathways supporting this have yet to be identified. Furthermore, since we do not yet have a full understanding of the genetic factors underlying VIPN development, new genetic pathways should be identified by use of recently developed methods of genetic testing. Some of these techniques, like next generation sequencing [67], have not yet been used in previous studies investigating the relation between VIPN and the genetic profiles of children. Therefore, future studies, in which these new techniques are applied, are needed.

However, one should be aware that usage of high-throughput genotyping techniques will produce massive amounts of data for testing genetic associations. Statistical analysis of these data needs to estimate many effects and test many hypotheses, and thus adjustments for multiple comparisons are needed [68]. In our review four out of 10 genetic studies reported to correct for multiple testing.

Studies investigating ways to decreasing VIPN in children are important as VIPN has been shown to be related to reduced quality of life, mainly caused by a decreased motor function or pain [13,17]. Moreover, a decrease in VIPN could lead to a decrease in medical costs. For example, it is known that 30% of children with ALL are referred to a physical therapist, which is mainly due to adverse effects of VCR [69]. Furthermore, it has been shown that chemotherapy-induced peripheral neuropathy is associated with a substantial increase in direct and indirect medical costs in adults [70]. However, the evidence regarding the relationship between VIPN in children and medical costs is scarce and this relationship should be studied in more detail.

The methodological quality of each of the studies included in this review was rated by computing a quality assessment score. In total, nine studies were rated as high-quality studies. In some cases, the number of studies that provided information on the relation between VIPN and a specific influencing factor was low. Omitting the low-quality studies would lead to the inability to report about the possible effect of this factor on VIPN. Therefore, our results were described in the light of the quality ratings of the studies as much as possible.

The results of this review has brought on some recommendations regarding future research of VIPN in children. First, in the current review it appeared to be rather difficult to compare study results due to the variety of tools for assessing VIPN. Many of these tools have limitations such as floor- and ceiling effects, moderate intra- and inter-rating reliability and lack of validity among children younger than five years. However, recently some new tools have been developed that do not have (some of these) limitations. For example, the ped-mTNS and the TNS-PV are tools that provide superior validity and inter- and intrarater reliability compared to other tools that have been widely used in the past, such as the CTCAE. The ped-mTNS and the TNS-PV are little time-consuming, inexpensive, non-invasive tools, of which the measurements can be performed by other non-physician healthcare professionals, such as physical therapists. Therefore, usage of these instruments in future studies investigating VIPN among children is recommended, thereby enabling the comparison of results across studies. However, the ped-mTNS and the TNS-PV can be used for children aged five years or older only. Therefore, alternative tools for VIPN assessment for children up to the age of five years should be developed.

Secondly, all studies included in the current review were either cohort or cross-sectional studies, while for studies investigating the relation between VIPN and certain patient-related factors such as age, gender and race, a randomized controlled

trial would the most appropriate study design to use. Moreover, none of the included studies were double-blinded studies, which increases the risk of observer bias.

Thirdly, it appeared that many of the studies focused on only one of the many factors that influence VIPN (e.g. genetic profiles, PK measures or race). This may have resulted in a one-sided perspective regarding the influence of that factor, without taking into account the (intervening) role of the other factors.

In addition, it should be noted that this review summarizes results from studies which included different diseases. It seems likely that differences in VIPN development between diseases exist. For instance, children with CNS tumors might also have motor- and sensory disorders, thereby making it difficult to distinguish between VIPN and other neurologic symptoms. Moreover, it should also be taken into account that these differences can be treatment-related. Radiation therapy, for instance, might increase the vulnerability to the nervous system for VIPN [71]. Furthermore can these differences be VCR-related, since treatment regimens differ regarding (cumulative) VCR dose, the interval between doses and treatment phase of VCR administration. Unfortunately, most of the studies included in our review investigated children with ALL. As a consequence, there was not sufficient power to study the role of disease type on VIPN. Therefore, studies should be undertaken that investigate multiple influencing factors of VIPN in specific disease types to expand our knowledge on these factors and their interplay.

Finally, the role of possible drug interactions, such as the azoles, was not reported in most of the included studies. Since it is known that azoles can have an interaction with VCR, probably due to the fact that these drugs are CYP inhibitors [34-36], this could have influenced the development of VIPN in several studies. Future studies should report on the medications which were given concomitant with VCR and which are known to interact with VCR and should take this into account when interpreting the results.

In conclusion, VIPN is a multifactorial type of VCR toxicity influenced by several variables such as age, race, genetic profile, dose and administration method. Older children and children of Caucasian race seem to be more susceptible to VIPN. Genetic profile, VCR dose and administration method all appear to influence the way VCR is metabolized, which in turn influences the susceptibility of a child to develop VIPN. This review provides a further understanding of what is known to date and which future studies are warranted in order to obtain a better understanding of the mechanism underlying VIPN. Ultimately, this will hopefully lead to the development

of personalized treatment for children with maximum therapeutic effectiveness while minimizing toxicity.

SUPPLEMENTARY MATERIALS

Supplemental Table S1: Literature search Pubmed

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Measuring vincristine-induced peripheral neuropathy in children with cancer: validation of the Dutch pediatric-modified Total Neuropathy Score

M.E. van de Velde*, S.M. Schouten*, G.J.L. Kaspers, L.B. Mokkink,
I.M. van der Sluis, C. van den Bos, A. Hartman, F.C.H. Abbink, M.H. van den Berg

* both authors contributed equally to this work

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ABSTRACT

Purpose. The aims were to evaluate the construct validity and reliability of the Dutch version of the pediatric modified total neuropathy score (ped-mTNS) for assessing vincristine-induced peripheral neuropathy (VIPN) in Dutch pediatric oncology patients aged 5-18 years.

Methods. Construct validity (primary aim) of the ped-mTNS was determined by testing hypotheses about expected correlation between scores of the ped-mTNS (range: 0-32) and the Common Toxicity Criteria for Adverse Events (CTCAE) (range: 0-18) for patients and healthy controls and by comparing patients and controls regarding their total ped-mTNS scores and the proportion of children identified with VIPN. Inter-rater and intra-rater reliability and measurement error (secondary aims) were assessed in a subgroup of study participants.

Results. Among the 112 children (56 patients and 56 age- and gender-matched healthy controls) evaluated**,** correlation between CTCAE and ped-mTNS scores was as expected (moderate (r=0.60)). Moreover, as expected, patients had significantly higher ped-mTNS scores and more frequently symptoms of VIPN compared to controls (both $p<.001$). Reliability as measured within the intra-rater group (n=10) (intraclass correlation coefficient ($ICC_{agreement}$)=0.64, standard error of measurement ($SEM_{agreement}$)=2.92 and smallest detectable change ($SDC_{agreement}$)=8.1 and within the inter-rater subgroup (n=10) ($ICC_{agreement}$=0.63 and $SEM_{agreement}$=3.7, $SDC_{agreement}$=10.26) indicate insufficient reliability.

Conclusion. The Dutch version of the ped-mTNS appears to have good construct validity for assessing VIPN in a Dutch pediatric oncology population, whereas reliability appears to be insufficient and measurement error high. To improve standardization of VIPN assessment in children, future research aimed at evaluating and further optimizing the psychometric characteristics of the ped-mTNS is needed.

INTRODUCTION

Vincristine (VCR) is a chemotherapeutic agent which is often used in pediatric oncology for the treatment of various hematological and solid cancers[1]. A frequently occurring side-effect of VCR is peripheral neuropathy[2]. VCR-induced peripheral neuropathy (VIPN) is a mixed sensory, motor and autonomous neuropathy mainly affecting the longer peripheral nerves. Symptoms of VIPN usually start in the distal part of the limbs and may progress proximally[3]. Symptoms include paresthesia, numbness, tingling, loss of proprioception and pain. Rarely, VIPN may lead to profound muscle weakness with symptoms as foot drop and walking difficulties. Autonomous symptoms of VIPN include constipation and dizziness[4]. VIPN showed to adversely affect quality of life in oncology patients[5].

VIPN is a multifactorial toxicity, which is influenced by several determinants. Older children seem to be more affected than younger children, whereas the influence of gender on VIPN is unclear[6]. Moreover, there is a racial difference: Caucasian children tend to have more VIPN than non-Caucasian children[7]. Furthermore, although there is a dose dependent relation between VCR and VIPN[8], studies assessing associations between pharmacokinetic parameters of VCR and VIPN report conflicting results[9,10]. Finally, genetic factors are known to influence susceptibility to VIPN, with several pathways involved such as CEP72 and CYP3A4/5[8,11].

Treatment of VIPN is mainly symptomatic using analgesics such as gabapentin, or laxatives in case of constipation. Another treatment option is dose reduction of VCR, although this may lead to suboptimal treatment of the underlying malignancy[12].

In clinical practice several tools are used to measure VIPN in pediatric oncology patients. The Common Terminology Criteria for Adverse Events (CTCAE) is a tool that assesses the severity of several types of adverse events in oncology patients[13], including those regarding peripheral neuropathy, constipation and neuralgia. Furthermore, Lavoie-Smith et al.[14,15] developed the Total Neuropathy Score-Pediatric Vincristine (TNS-PV) specifically for the assessment of VIPN in pediatric oncology patients. The TNS-PV consists of a seven-item interview-based questionnaire and a standardized physical examination (testing of vibration and temperature sense, muscle strength and deep tendon reflexes (DTR))[6]. Besides questionnaires and/or physical examinations, nerve conduction studies in which the conduction velocity of different nerves is measured using somatosensory evoked potentials[16,17] can also be used to assess VIPN.

However, all of the above-mentioned methods to assess VIPN have some limitations. Although frequently used, the CTCAE shows insufficient sensitivity in detecting motor- and sensory neuropathy[2]. Moreover, the TNS-PV can only be used in children aged six years or older[18]. Furthermore, nerve conduction studies are invasive, painful and expensive[18]. Finally, physical examination can only validly be performed and interpreted by specifically trained physicians such as pediatric neurologists[19,20].

Recently, the pediatric modified-Total Neuropathy Score (ped-mTNS) was developed[21]. This instrument, which consists of an interview-based questionnaire and physical examination, showed to have superior psychometric characteristics compared to other tools for the assessment of peripheral neuropathy in children[2]. Moreover, it is a quick, inexpensive and non-invasive tool that can be employed by different health care professionals, such as physicians, physical therapists and nurses[4]. However, the psychometric characteristics of the ped-mTNS have solely been evaluated in North-American children with cancer aged 5-18 years.

The primary aim of the current study was to evaluate the construct validity of the Dutch version of the ped-mTNS for the assessment of VIPN in Dutch pediatric oncology patients aged 5-18 years. In addition as secondary aim, reliability of this tool was investigated in a subpopulation.

METHODS

Study population

The study population of this multicenter cross-sectional study consisted of pediatric oncology patients aged 5-18 years with non-CNS malignancies and healthy controls.

Patients were eligible for study participation if they were treated with at least four administrations of at least 1.5 mg/m^2 (maximum 2 mg) VCR within a period of six weeks during treatment of their current malignancy. VIPN assessments were performed within the time frame of at least one month after start of VCR therapy and two months after cessation of VCR therapy maximally. This resulted in the inclusion of patients with the following diagnoses and treatment protocols for the current study: acute lymphoblastic leukemia (ALL) (DCOG ALL-11 protocol[22]), Hodgkin lymphoma (EuroNet-PHL-C1 protocol[23] or C2 protocol[24]), nephroblastoma (SIOP Wilms 2001 protocol[25]) and rhabdomyosarcoma (EpSSG RMS 2005 protocol[26]). VCR was administered either by means of a bolus injection (1-5 minutes) or a one-hour infusion. The healthy control group consisted of siblings of participating patients and children known to hospital co-workers (relatives or friends). Controls were age- (± 1 year) and gender-matched to the patients on a 1:1 basis. Patients and controls were excluded in case of premorbid developmental disorders, neuromuscular disorders, lower extremity amputations, diabetes mellitus, or in case they were not able to speak or understand the Dutch language. Although strictly speaking the included controls could not develop VIPN (as they did not receive any VCR), hypothetically they may have some degree of peripheral neuropathy. The ped-mTNS is a tool which assesses peripheral neuropathy, either chemotherapy-induced or not, in children. For reasons of clarity, we use the term VIPN in the remainder of this paper, thereby referring to either VCR-induced (patients) or non-VCR-induced (controls) peripheral neuropathy.

Compliance with Ethical Standards

The Medical Ethics Review Committee of Amsterdam UMC location VUmc determined that the Medical Research Involving Human Subjects Act (WMO) did not apply to this study. There are no potential conflicts of interest. Informed consent was obtained from all parents of participating children and from participants themselves in case they were 12 years or older.

Ped-mTNS

The original, English version of the ped-mTNS was translated into Dutch by a non-native English speaker, followed by a translation back to English by a bilingual Dutch-English speaker. Subsequently, this back-translated version was sent to the principal

investigator of the original ped-mTNS in the USA[4] in order to have it reviewed and checked by its original developer. Appendix A and B contain the original version and the Dutch version of the ped-mTNS as approved by the developer, respectively.

The questionnaire-part of the ped-mTNS contains eight questions about sensory, functional and autonomic symptoms. These questions were read out aloud by the assessor to the participant. In addition, five different aspects of VIPN were assessed by physical examination: light-touch sensation by Semmes-Weinstein monofilaments (Rolyan–Ability One, Germantown, WI, USA)[4,27], pin sensibility by Medipin™ (Ltd, Hertfordshire, UK)[4], muscle strength by means of manual muscle testing (graded according to the Medical Research Council guidelines)[4,28], and DTR of the Achilles and patella (graded according to the Mayo Clinic Criteria)[4,29]. Due to the unavailability of a Biothesiometer™ (Biomedical Instruments, Newbery OH, USA) in the Netherlands, the assessment of vibration sense was carried out with a Rydel-Seiffer 64 Hz tuning fork (Gebrueder Martin, Tuttlingen, Germany), which showed to be a valid instrument in the assessment of vibration sense in children[4,30]. For all items in the questionnaire and the physical examination part, the score ranged between zero (no symptoms) and four (severe symptoms). The worst scores within each of the three items as assessed by the questionnaire, together with the scores of the five items tested in the physical examinations, are used to calculate the total ped-mTNS score, which is the sum of these eight scores (range 0 to 32). Children with a total ped-mTNS score of five or higher were considered to have VIPN[4].

CTCAE

The CTCAE (version 4.03)[13] consists of over 330 items scoring adverse events due to cancer treatment divided into 26 different categories. Possible grades range from zero (no symptoms) and three (severe symptoms) or, if the adverse event can be deadly, category four is life threatening and category five is death. The CTCAE items used for the assessment of VIPN are constipation, peripheral sensory neuropathy, peripheral motor neuropathy and neuralgia. The maximum CTCAE sum score of these four items is 18. Participants with a total score of two or higher are considered to have VIPN[6]. The assessor asked to which extent the participants experienced problems as described in the relevant CTCAE items. For the item peripheral sensory neuropathy, additionally DTR of the patella and Achilles were assessed.

Procedures

Patients were included in Emma Children's Hospital/Amsterdam University Medical Centers and Sophia Children's Hospital/Erasmus Medical Center Rotterdam. Study measurements were performed between September 2016 and July 2017. All study

participants (patients and controls) were tested once using both the ped-mTNS and CTCAE to assess VIPN. These measurements were performed by the same assessor (SS), who was trained extensively by a pediatric neurologist to perform the VIPN assessments. Furthermore, a subset of randomly selected patients and healthy controls were assessed twice in order to assess intra-rater ((5 patients and 5 controls), both measurements performed by the same assessor) and inter-rater reliability ((7 patients and 3 controls) second measurement performed by a different assessor (MvdV), who was also specifically trained for the study measurements). All patients were measured during regular hospital visits. Healthy controls were measured either during family visits in the hospital or at home. For the assessment of intra-rater reliability, an interval of 4-16 days between the two measurements, without VCR administrations in between, was adhered to.

Statistics

Assessment of construct validity was determined by calculating the correlation between the ped-mTNS and the CTCAE sum scores in patients as well as the actual differences between these scores. We hypothesized total ped-mTNS and total CTCAE scores to be moderately correlated, since some items between the two systems attempt to measure the same items (peripheral motor neuropathy and peripheral sensory neuropathy), whereas some items are only present in one of the instruments (constipation is only an item in the CTCAE and not in the ped-mTNS). Moreover, we expected to assess a higher correlation between the two measurement tools than the correlation measured in the study of Gilchrist et al. ($r=0.07$)[2], since in that study the CTCAE scores were retrieved from medical records instead of the prospective assessment of CTCAE scores used in our study. Either Spearman or Pearson correlation coefficients were calculated, depending on normality of data distribution. According to Nunnally and Bernstein[31] an ICC of >0.70 was considered as good. Furthermore, a correlation of 0.51-0.69 was considered moderate and <0.5 as low[32]. We hypothesized ped-mTNS scores of patients to be significantly higher compared to healthy controls and the proportion of patients with VIPN to be significantly higher than those of healthy controls. Differences between mean total ped-mTNS score of patients and controls were analyzed using a Mann-Whitney U test. Differences between the proportion of patients and controls who were identified as having VIPN by the ped-mTNS were calculated using a Chi-square test. A two-tailed significance level of 0.05 was used.

Assessment of reliability and standard error of measurement of the ped-mTNS (secondary aims) were carried out as follows. Reliability and measurement error were measured within the inter-rater and intra-rater subgroups by means of intraclass correlation coefficient using the two-way random effects model for

agreement ($ICC_{agreement}$) according to the equation $ICC_{agreement} = \frac{{\sigma_p}^2}{{\sigma_p}^2+{\sigma_o}^2+{\sigma_{residual}}^2}$ where ${\sigma_{error}}^2 = {\sigma_o}^2 + {\sigma_{residual}}^2$[33]. Measurement error was assessed by calculating the Standard Error of Measurement from the same model (i.e. the two-way random effects model for agreement) ($SEM_{agreement}$) by the equation $SEM_{agreement} = \sqrt{({\sigma_o}^2 + {\sigma_{residual}}^2)}$ and by calculating the Smallest Detectable Change ($SDC_{agreement}$) using the equation $SDC_{agreement} = 1{,}96 \times \sqrt{2} \times SEM_{agreement}$[34,35].

Dichotomized total scores of the ped-mTNS (yes/no VIPN; a total ped-mTNS score of 0-4 indicates no VIPN and a score of ≥5 indicates VIPN according to published data by Gilchrist et al.[4]) were used to measure agreement in a 2x2 table to calculate positive agreement (PA) (PA=2*a*/(2*a*+*b*+*c*) and negative agreement (NA) (NA=2*d*/(2*d*+*b*+*c*).

All results on reliability are reported for patients, healthy controls, and total group separately. All statistics were performed using SPSS for Windows version 22.0 (SPSS, Chicago, IL).

Table 1. Baseline characteristics of study participants

	Patients (n=56)[1]	**Healthy controls (n=56)[1]**
Sex		
Male	32 (57.1)	32 (57.1)
Female	24 (42.9)	24 (42.9)
Age in years	9.7 (6.3-14.1)	9.6 (7.0-14.4)
Ethnicity		
European	40 (71.4)	39 (69.6)
Middle-Eastern	10 (17.9)	6 (10.7)
African	1 (1.8)	4 (7.1)
Hispanic		4 (7.1)
Other	5 (8.9)	3 (5.4)
Diagnosis[2]		
ALL	39 (69.6)	
Hodgkin lymphoma	12 (21.4)	
Nephroblastoma	1 (1.8)	
Rhabdomyosarcoma	4 (7.1)	
Administration method of VCR[2]		
Bolus injection	47 (83.9)	
1-hour infusion	9 (16.1)	
Cumulative dose of VCR (mg/m²; mean (SD))[2]	19.6 (13.91)	
Time since last VCR dose (days)[2]	9.5 [0.75-21.0]	

[1]Values represent the number (%) of participants, unless indicated otherwise
CNS central nervous system, *IQR* interquartile range, *ALL* acute lymphoblastic leukemia, *RMS* rhabdomyosarcoma, *LGG* low-grade glioma, *VCR* vincristine, *ped-mTNS* pediatric-modified Total Neuropathy Score, *CTCAE* Common Terminology Criteria for Adverse Events., [2]This is only applicable to the patient population.

RESULTS

Study population

Fifty-six patients and 56 healthy controls (median (IQR) age 9.6 (6.6-14.2) years) were included in the study. Characteristics of the participants are presented in Table 1. The majority of the patients were treated for ALL (n=39, 70%) or Hodgkin lymphoma (n=12, 21%). The mean (SD) cumulative dose of VCR administered to the patients was 19.6 mg/m^2 (13.9).

Construct validity of the ped-mTNS

The correlation between total scores of the ped-mTNS and CTCAE was moderate as expected in patients (r=0.60). Patients had a significantly higher score on ped-mTNS than healthy controls (median (IQR): 10.0 (6.25-13.0)) and (median (IQR): 0.0 (0.0-1.0), respectively p<.001) (Table 2). Furthermore, patients were significantly more often identified as having VIPN (≥5 on the ped-mTNS) than healthy controls (86% versus 1.8%, respectively p<.001).

Table 2. Results of peripheral neuropathy measurements in the two study groups

	$ICC_{agreement}$ total ped-mTNS score	95% CI ICC total ped-mTNS score	$SDC_{agreement}$	$SEM_{agreement}$	Positive agreement of total ped-mTNS score	Negative agreement of total ped-mTNS score
Inter-rater reliability patients (n=7)	0.45	-0.43 – 0.88	12.25	4.42	100%	100%
Inter-rater reliability healthy controls (n=3)	0	-	5.73	2.07	-	100%
Intra-rater reliability patients (n=5)	0	-1.3 – 0.20	8.96	3.23	80%	0%
Intra-rater reliability healthy controls (n=5)	0	-	0	0	-	100%
Inter-rater reliability total (n=10)	0.63	0.04 – 0.89	10.26	3.7	100%	100%
Intra-rater reliability total (n=10)	0.64	0.06 – 0.90	8.1	2.92	80%	83%

ICC=intraclass correlation coefficient, ped-mTNS: pediatric modified total neuropathy score, CI: confidence interval

Reliability

All results regarding reliability for patients, healthy controls and the total group of participants are depicted in Table 3. The different variance components of the intra-rater reliability group were: σ_p^2=15.3, σ_o^2=0 and $\sigma_{residual}^2$=8.6 and of the inter-rater reliability: σ_p^2=23.1, σ_o^2=0 and $\sigma_{residual}^2$=13.7. The $ICC_{agreement}$ of the intra-rater group was 0.64, whereas this was 0.63 of the inter-rater group. Positive agreement was 80%, negative agreement was 83%. Positive and negative agreement of inter-rater reliability were both 100%. Finally, the $SEM_{agreement}$ within the intra-rater group was 2.92 and within the inter-rater group 3.7. The $SDC_{agreement}$ was 8.1 within the intra-rater group and 10.26 within the inter-rater group.

Table 3. Reliability outcome measures of the pediatric modified total neuropathy score

	Patients (n=56)	Healthy controls (n=56)	p-value
Total ped-mTNS scores			
Median (IQR)	10.0 (6.25-13.0)	0.0 (0.0-1.0)	<0.001*
Score of 0-4 (n, %)	8 (14.3)	55 (98.2)	
Score 5-32 (n, %)	48 (85.7)	1 (1.8)	
Total CTCAE scores			
Median (IQR)	3 (2-4.75)	0 (0-0)	<0.001*
Score of 0-1	9 (16.1)	55 (98.2)	
Score of 2-4	33 (59.0)	1 (1.8)	
Score of 5-7	12 (21.4)	0 (0)	
Score of 8-18	2 (3.6)	0 (0)	

ped-mTNS pediatric-modified Total Neuropathy Score, *CTCAE* Common Terminology Criteria for Adverse Events, *results are statistically significant

DISCUSSION

In this study the Dutch version of the ped-mTNS was validated by assessing VIPN in a cohort of Dutch pediatric oncology patients aged 5-18 and age- and gender-matched healthy controls. The correlation between ped-mTNS and CTCAE was found to be as expected (i.e. moderate). Furthermore, patients had significantly higher ped-mTNS scores than controls and were significantly more often identified as having VIPN. These results indicate that this translated version of the ped-mTNS has good construct validity regarding the assessment of VIPN in Dutch pediatric oncology patients. However, reliability of this instrument was insufficient ($ICC_{agreement}$ < 0.7). The outcomes of measurement error showed a $SEM_{agreement}$ of 2.92 and 3.7 and a $SDC_{agreement}$ of 8.1 and 10.26 for the intra-rater and inter-rater subgroups, respectively. Although the minimal important change (MIC) of this instrument is unknown, these $SEM_{agreement}$ and $SDC_{agreement}$ scores appear to be rather high, given the fact that scores can range from 0 to 32 and we used a cutoff value of ≥5 to discriminate children with and without VIPN. However, positive and negative agreement were good, with scores between 80% and 100% for intra-rater and inter-rater reliability, respectively.

The results of the current study are comparable to a previous study. Gilchrist et al.[4] recently showed that patients had a significantly higher score on ped-mTNS than healthy controls (mean (SD): 8.7 (4.2); and 1.4 (0.9, respectively). However, in that study only 9.8% of the controls had a score of zero on the ped-mTNS, while this percentage was 68% in the current study. This discrepancy can most likely be attributed to differences in carrying out the assessments with the ped-mTNS, such as the level of training of the assessors, and not to a difference in population. The results of Gilchrist et al. showed that healthy controls frequently experienced some disorders of motor function, autonomic symptoms, pin sensation and distal strength, although in both studies scores of healthy individuals were not high enough to indicate VIPN (ped-mTNS score was < 5) and therefore not of great clinical relevance. In the current study, the healthy control group had a mean (SD) ped-mTNS score of 0.9 (1.5). In the study of Gilchrist et al. similar scores were reported (mean (SD) 1.4 (0.9)). Furthermore, the current study showed an $ICC_{agreement}$ score of the ped-mTNS for intra- and inter-rater reliability of 0.64 and 0.63, respectively. These scores are lower than the ICC sores for intra-rater and inter-rater reliability as reported by Gilchrist et al. (0.99 and 0.98, respectively)[4]. This may be due to the fact that within the study of Gilchrist et al. an interval of only one hour between two measurements was applied when assessing inter- and intra-rater reliability, whereas in our study this interval was four days minimally. An interval of one-hour may have led to recall bias of the patient for the interviewed questions and of the assessor for the physical examination part, since there is a high chance

that the results of the previous measurement are memorized. Furthermore, Gilchrist et al. have used a two-way mixed effect model for consistency for their calculation of ICC (i.e. $ICC_{consistency}$), therefore the variance due to systematic differences between observers was ignored[33,34]. By using $ICC_{agreement}$, which we did in our study, this variance of observers was taken into account. However, using $ICC_{consistency}$ instead of $ICC_{agreement}$ leads to higher ICC values, which could be an explanation for the difference in results between these studies[36].

Our study was the first to evaluate psychometric characteristics of a translated version of the ped-mTNS. Our results are important both for clinical purposes and research practice as they contribute to the urgently needed standardization of measuring peripheral neuropathy in children with cancer using high quality outcome measurement instruments.

This study has some limitations. As previously mentioned, the Rydel-Seiffer 64 Hz tuning fork was used for the assessment of vibration sense instead of the Biothesiometer™, due to unavailability of this device in the Netherlands. However, according to Hilz et al.[30] the Rydel-Seiffer 64 Hz tuning fork is a valid instrument to examine vibration sense and Gilchrist et al.[21] showed a moderate to good correlation (i.e. r = -0.62 - -0.73, depending on test site) between the Biothesiometer™ and the Rydel-Seiffer 64 Hz tuning fork.

The assessment of reliability and measurement error were only evaluated within a subgroup of 20 participants. Although there is no formal consensus about the minimal number of participants needed to properly asses the inter- and intra-rater reliability[37], the number of participants included for these assessments are probably rather low as can be seen in the 95% confidence intervals of the reliability estimations, due to logistical limitations. Therefore, these results should be interpreted with caution. Future studies assessing the reliability of the ped-mTNS in a larger group of patient are advocated. These studies can be performed within a patient population, since results regarding reliability of healthy controls will probably be difficult to interpret due to floor effects.

Despite certain psychometric limitations, the ped-mTNS is currently considered to be the most optimal instrument for assessing VIPN in children compared to other instruments[38]. Continuous efforts should be made to further improve this instrument, by studying and advancing its reliability and by additionally assessing of its validity. Specifically, more research should be undertaken to investigate the content validity of this instrument, by assessing if the ped-mTNS is complete in measuring all the

aspects of VIPN in terms of relevance and comprehensibility of this instrument, both for patients and assessor. Finally, it should be investigated to which extent an adapted version of the ped-mTNS could be developed that is suitable for assessing VIPN in children under the age of five. All above-mentioned efforts may result in a more valid and reliable instrument for the assessment of VIPN in children. Meanwhile, using the current version of the ped-mTNS is advocated since it will lead to more uniformity in assessing VIPN in children with cancer, thereby enabling the comparison of study results for this group of patients [38].

In conclusion, the current study showed that the Dutch translated version of the ped-mTNS has a good construct validity, whereas reliability appeared insufficient, although patients numbers for reliability testing were low. In order to improve the comparability of results across different studies investigating VIPN in children, further standardization of VIPN assessment is needed. More research aimed at investigating and improving the quality of the ped-mTNS, or in the development of another instrument to assess VIPN, is needed. This will ultimately lead to a robust instrument and more uniformity in evaluating chemotherapy induced peripheral neuropathy in children with cancer.

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APPENDIX A. ORIGINAL VERSION OF THE PEDIATRIC-MODIFIED TOTAL NEUROPATHY SCORE

Sensory Symptoms:____ (record worst score for the three sensations)

"Do you have any parts of your body that are tingly, numb (can hardly feel), or hurt?"

____ Tingly ____ Numb ____ Hurt (record number for each)

If yes, "Where you have those feelings?"

0 None
1 Symptoms limited to fingers or toes
2 Symptoms extend to ankles or wrists
3 Symptoms extend to knee or elbow
4 Symptoms above knee or elbow

Functional Symptoms:____ (record worst score of the three questions)

"Do you have trouble buttoning shirts or zipping zippers?" ____

"Do you have trouble walking such as tripping frequently?" ____

"Do you have trouble going up or down stairs?" ____

If yes to any, "Is it....(read choices)" and record after each question

0 Not Difficult
1 A little difficult
2 Somewhat difficulty
3 I need help
4 I can't do that at all

Autonomic Symptoms:____ (record worst score of the three questions)

"Do you feel dizzy or light-headed when you get up out of bed?" ____

"Do your hands or feet feel hotter or colder than normal?"

0 Never
1 A little bit
2 Sometimes
3 Very much
4 Almost always

Semmes	Semmes
Toes R	Finger R
L	L
Med Mal R	Wrist R
L	L
Knee R	Elb R
L	L

Clinical Testing :

Light Touch Sensation: ____

0 Normal
1 Reduced in fingers/toes
2 Reduced up to wrist/ankle
3 Reduced up to elbow/knee
4 Reduce to above elbow/knee

Pin Sensibility: ____

0 Normal
1 Reduced in fingers/toes
2 Reduced up to wrist/ankle
3 Reduced up to elbow/knee
4 Reduce to above elbow/knee

Bioesth	Bioesth
Toes R	Finger R
L	L
Med Mal R	Wrist R
L	L
Knee R	Elb R
L	L

Vibration Sensibility:____ (worst score)

0 Normal
1 Reduced in fingers/toes
2 Reduced up to wrist/ankle
3 Reduced up to elbow/knee
4 Reduced to above elbow/knee

Strength: ____ Worst Score (MRC Score R / L)

MRC level: Great Toe ____/____Ankle DF____/____Finger abd____/____ Wrist ext____/____

0 Normal

1 Mild weakness (MRC 4)

2 Moderate weakness (MRC 3)

3 Severe weakness (MRC 2)

4 Paralysis (MRC 1-0)

DTR: ____ (Achilles, Patellar)

0 Normal

1 Ankle reflex reduced (Achilles +1)

2 Ankle reflex absent (Achilles 0, Patellar +2)

3 Ankle reflex absent, others reduced (Achilles 0, Patellar +1)

4 All reflexes absent (all 0)

Total Score: ____/ 32

APPENDIX B. DUTCH VERSION OF THE PEDIATRIC-MODIFIED TOTAL NEUROPATHY SCORE

Interview vragen

Vraag sensorische symptomen

Zijn er bepaalde lichaamsdelen die tintelen, verdoofd voelen (die je bijna niet kan voelen), of pijn doen?

____Tintelingen ____Verdoofd ____Pijn (noteer aantal voor elke sensatie)

Zo ja, *Waar heb je dat gevoel?*

0 Nergens
1 Alleen symptomen in vingers of tenen
2 Symptomen uitgebreid tot enkels of polsen
3 Symptomen uitgebreid tot knie of elleboog
4 Symptomen tot boven de knie of elleboog

Score sensorische symptomen:____ (noteer de slechtste score van de drie sensaties)

Vragen functionele symptomen

Heb je problemen met het dichtknopen van een blouse of dichtritsen van je rits? Is dit ..... (lees keuzemogelijkheden voor):

0 Niet moeilijk
1 Een beetje moeilijk
2 Enigszins moeilijk
3 Ik heb hier hulp bij nodig
4 Ik kan dat helemaal niet

Heb je problemen met lopen? (struikel je bijvoorbeeld vaak?) Is dit ..... (lees keuzemogelijkheden voor):

0 Niet moeilijk
1 Een beetje moeilijk
2 Enigszins moeilijk
3 Ik heb hier hulp bij nodig
4 Ik kan dat helemaal niet

Heb je problemen met de trap op of af lopen? Is dit ..... (lees keuzemogelijkheden voor):

0 Niet moeilijk
1 Een beetje moeilijk

2 Enigszins moeilijk
3 Ik heb hier hulp bij nodig
4 Ik kan dat helemaal niet

Functionele symptomen:____ (noteer de slechtste score van de drie vragen)

Vragen autonome symptomen

Voel je je duizelig of licht in je hoofd wanneer je opstaat uit bed?

0 Nooit
1 Een beetje
2 Soms
3 Heel erg
4 Bijna altijd

Voelen je handen of voeten warmer of kouder aan dan normaal?

0 Nooit
1 Een beetje
2 Soms
3 Heel erg
4 Bijna altijd

Autonome symptomen:____ (noteer de slechtste score van de twee vragen)

Neurologisch onderzoek

Semmes	Semmes
Tenen R	Vinger R
L	L
Med Mal R	Pols R
L	L
Knie R	Elleboog R
L	L

Lichte Tast sensatie:____

0 Normaal
1 Verminderd in vingers/tenen
2 Verminderd tot aan de pols/enkel
3 Verminderd tot aan de elleboog/knie
4 Verminderd tot boven de elleboog/knie

Pijnzin:____

0 Normaal
1 Verminderd in vingers/tenen
2 Verminderd tot aan de pols/enkel
3 Verminderd tot aan de elleboog/knie
4 Verminderd tot boven de elleboog/knie

Vibratiezin:____

Rydel	Rydel
Tenen R	Vinger R
L	L
Med Mal R	Pols R
L	L
Knie R	Elleboog R
L	L

0 Normaal
1 Verminderd in vingers/tenen
2 Verminderd tot aan de pols/enkel
3 Verminderd tot aan de elleboog/knie
4 Verminderd tot boven de elleboog/knie

Kracht:____ Slechtste Score (Medical Research Council (MRC) Score R / L)
MRC level: Grote teen___/___Enkel dorsoflexie___/___Vinger abductie___/___Pols extensie___/___

0 Normaal
1 Milde zwakte (MRC 4)
2 Matige zwakte (MRC 3)
3 Ernstige zwakte (MRC 2)
4 Paralyse (MRC 1-0)

Reflexen:____ (Achillespees, kniepees)

0 Normaal
1 Verminderde achillespeesreflex (Achilles -1 t/m-3 OF +1)*
2 Afwezige achillespeesreflex (Achilles -4, kniepeesreflex 0 OF Achilles 0, kniepeesreflex +2)*
3 Afwezige achillespeesreflex, verminderde kniepeesreflex (Achilles -4, kniepeesreflex -1 t/m -3 OF Achilles 0 kniepeesreflex +1)*
4 Alle reflexen afwezig (allemaal-4 OF allemaal 0) *

* *Afhankelijk van gebruikte scoringsmethode*

The association between vincristine-induced peripheral neuropathy and health-related quality of life in children with cancer

M.E. van de Velde, M. H. van den Berg, G.J.L. Kaspers, F.C.H. Abbink, J.W.R. Twisk, I.M. van der Sluis, C. van den Bos, M.M. van den Heuvel – Eibrink, H. Segers, C. Chantrain, J. van der Werff Ten Bosch, L. Willems and R.R.L. van Litsenburg

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ABSTRACT

Purpose Vincristine (VCR) is a chemotherapeutic agent used in the treatment of pediatric oncology patients, but its main toxicity is VCR-induced peripheral neuropathy (VIPN). However, whether VIPN has an effect health-related quality of life (HR-QoL) in children during treatment is unknown. Therefore, the aim of our study was to investigate the association between VIPN and HR-QoL in children starting treatment for cancer.

Methods Measurements of VIPN were performed using two tools: Common Terminology Criteria for Adverse Events (CTCAE) and pediatric-modified Total Neuropathy Score (ped-mTNS). Assessment of HR-QoL was done with self- and proxy assessment of the Cancer and Generic module of the Pediatric Cancer Quality of Life Inventory™ (PedsQL).

Results N=86 children were included. HR-QoL of children with VIPN (n=67, 76%) was significantly lower in comparison with children without VIPN: estimated Total score of PedsQL Generic (proxy) 84.57; β=-8.96 and 95% confidence interval (CI) -14.48 to -3.43; p=0.002, estimated PedsQL Generic Total score (self-reported): 85.16, β=-8.38 (95% CI: -13.76 to -3.00); p=0.003. Similar results were found in the Pain and Hurt domain of the PedsQL Cancer (pain: estimated score (proxy): 85.28, β=-9.94 (95%CI: -16.44 to -3.45), p=0.003; hurt: estimated score (self-report) 97.57, β=-19.15 (95%CI: -26.82 to -11.48), p<0.001).

Conclusion VIPN results in a significant reduction of HR-QoL in children under treatment for a malignancy, which means that VIPN is important for the well-being of pediatric oncology patients. Therefore, this study underlines the importance of optimizing treatment with VCR, thereby aiming to reduce VIPN while maintaining efficacy.

INTRODUCTION

Since the 1950's, survival of pediatric cancer gradually increased with currently an overall 5-year survival rate of approximately 80%[1]. However, 25% of this population experiences a severe or life-threatening disability related to the oncologic treatment[2]. Besides the increased incidence of disabilities, the treatment of a malignancy in childhood also leads to a reduction in health-related quality of life (HR-QoL) compared to healthy peers and siblings[3]. Moreover, this reduced HR-QoL is found in pediatric patients during anticancer treatment, but also in survivors of childhood cancer[4].

HR-QoL constitutes of effect of illness, treatment and health on important domains of life, such as physical, psychological, and social domains[5,6]. Reduced HR-QoL during anti-cancer treatment in children has been reported in all major domains[7,8]. Risk factors for impaired HR-QoL in childhood cancer include (mal-)adjustment to chronic illness in children, which can be explained by the disability-stress-coping model[9]. This model describes risk- and protective factors that influence the ability to adjust to a chronic disease[9]. Furthermore, HR-QoL is related to diagnosis[10]. Finally, HR-QoL depends on co-treatment of specific agents, such as glucocorticoids[11].

Vincristine (VCR) is a chemotherapeutic agent that is often used in children with cancer. Unfortunately, VCR-induced peripheral neuropathy (VIPN) leads to limitations in the maximum tolerable dose. Symptoms include paresthesia, constipation, muscle weakness, areflexia, neuropathic pain, and loss of sensibility[12]. The development of VIPN during treatment depends on several factors[13]. Treatment is symptomatic and includes the use of analgesics such as gabapentin and amitriptyline. However, the only effective treatment option for VIPN is dose-reduction of VCR, even though this hampers optimum treatment[14]. Alternatively, prolongation of VCR administration duration can be used even though evidence of efficacy is scarce[15,16].

It was shown that in adults, chemotherapy-induced peripheral neuropathy is associated with reduced HR-QoL during and after treatment[17]. Furthermore, we know that during cancer treatment pain has an impact on HR-QoL[18]. However, to what extent VIPN impacts HR-QoL in children with cancer during treatment, is unknown.

Therefore, we studied the impact of VIPN during treatment of various types of malignancies on HR-QoL in children during the first 12 months of treatment.

METHODS

Patients

This study is part of a prospective trial that studied the relation between infusion duration of VCR and the development of VIPN in pediatric oncology patients (the VINCA trial)[16]. This trial was registered in the Dutch Trial Registry (www.trialregister.nl; trial number NL4019). Participants of this trial received all their VCR administrations throughout treatment either as a push-injection or as an one-hour infusion. In total, ten treatment centers in the Netherlands and Belgium (n=6 in Belgium, n=4 in the Netherlands) were open for patient inclusion. Patients in this study were newly diagnosed children with cancer, starting VCR therapy and were between 2-18 years of age.

Children with different diagnoses and corresponding treatment protocols were included in this trial. The majority were hematological patients with either acute lymphoblastic leukemia (treated according to DCOG ALL-11 protocol[19], EsPhALL protocol[20], or EORTC-58081-CLG guideline[21]) or Hodgkin's lymphoma (treated according to EuroNet-PHL-C1 protocol[22] or C2 protocol[23]). Furthermore, solid tumor patients were included with nephroblastoma (treated according to SIOP Wilms 2001 protocol[24]) or rhabdomyosarcoma (treated according to EpSSG RMS 2005 protocol[25]). Finally, children with the following central nervous system (CNS) tumors were included: low-grade glioma (LGG) (treated according to SIOP-LGG 2004[26] protocol) and medulloblastoma (treated according to ACNS0331[27] protocol or ACNS0332[28] protocol). Children with CNS tumors were only eligible if at diagnosis they did not experience any sensory or motor symptoms of their limbs. Finally, due to the difficulties in assessing VIPN, patients with either pre-existent peripheral neuropathy or mental retardation could not participate in the trial.

This trial was conducted according to the principles of the Declaration of Helsinki. Furthermore, this trial adhered to all applicable country-specific regulatory guidelines. Before trial participation, informed consent in writing was collected from guardians or parents of all trial participants between 2-17 years. Furthermore, children who were aged between 12-17 years provided informed consent in writing themselves. The informed consent form and the study protocol received approval from the Institutional Review Board of Amsterdam UMC, location VUmc and were also approved at each of the trial sites.

Trial regimen

In the current report the results are reported of data collected during the first year after diagnosis. During this year, we measured HR-QoL and VIPN between 1 and 3 times. The number of measurements depended on the amount of VCR administrations

and duration of treatment (and treatment group). On average, VIPN and HR-QoL were assessed every 3 months.

Assessment of VIPN

Both VIPN and HR-QoL were assessed simultaneously on the same day of treatment. An overview of timing of measurements per disease type is shown in Supplemental Figure S2. For the measurement of VIPN, two different measurement tools were used. The CTCAE items (version 4.03)[29] peripheral motor neuropathy (score range: 0–5), peripheral sensory neuropathy (score range: 0–5), neuralgia (score range: 0–3), and constipation (score range: 0–3) were used. These item scores were summed into a total VIPN score. Furthermore, the dichotomized definition of VIPN was an item score of ≥2. In addition, the ped-mTNS (Dutch translation) was used for VIPN assessment[30]. This validated instrument for assessing VIPN in children includes a questionnaire (functional, sensory and autonomic symptoms) and a physical examination. It can be used for VIPN assessment in pediatric patients aged five years or older. Therefore, in our study we only used ped-mTNS assessment in the corresponding age group. As previously published, a total ped-mTNS score of ≥5 indicated that the child had VIPN[31].

Assessment of health-related quality of life

Assessment of HR-QoL was done by using the Dutch translated Generic Pediatric Cancer Quality of Life Inventory™ (PedsQL) (version 4.0) and the Acute Cancer PedsQL (version 3.0) (both 1 week recall)[32-34]. Both parent/guardian-proxy (all children) and self-reported (aged ≥5 years) questionnaires were used. The PedsQL generic version has 23 items, which can be summed to a total scale score. Additionally, there are two other sum-scores: psychosocial and physical. Individual items were scored on a Likert scale (5 points). However, visual scales (3-points) that use sad, neutral and happy faces were used for the responses of the children aged 5-7 years group. These require an interviewer for assessment. Score range is 0-100, in which a higher scores means a better HR-QoL. The PedsQL Acute Cancer is a reliable 27-item multidimensional cancer-specific questionnaire[35]. Subscales determine problems in different important areas during cancer treatment. It was hypothesized that VIPN can lead to diminished scores on the Pain and Hurt item, but is less likely to affect the other domains that are tested with the PedsQL Cancer questionnaire. Therefore, only the Pain and Hurt domain of the PedsQL Cancer was reported. Similar to the Generic version, the PedsQL Cancer also uses Likert scales (5 points) for item scores. These scores can range from 0 to 100, with higher scores being indicative for better HR-QoL.

Statistics

Normally distributed descriptive data were reported as means (standard deviation (SD)). Similarly, skewed variables of descriptive data were reported as medians (inter-

quartile range (IQR)). Patient characteristics of patients without and with VIPN were analyzed using a chi-square test or an independent t-test. Participants were identified with VIPN if they fulfilled the CTCAE or the ped-mTNS criteria specified above at least once during follow-up.

Differences in HR-QoL between participants with and without VIPN were evaluated using linear mixed model analyses of PedsQL outcomes. Subgroups were analyzed for patients who had VIPN according to either ped-mTNS *or* to CTCAE. First, the relation between PedsQL scores and VIPN was analyzed univariately, but since VIPN and HR-QoL are multifactorial, influenced by several factors, such as sex and age[13,36], we also used a forward selection procedure, to study variables that potentially influence differences in HR-QoL (PedsQL generic total score and PedsQL Cancer Pain and Hurt scale) between participants with and without VIPN. The following variables were evaluated: randomization, diagnosis, sex, age, cumulative VCR dose per m^2, use of analgesics, time since diagnosis and ethnicity. Analyses were done in SPSS 26.0 (Chicago, USA). A p-value of <0.05 was used as cutoff value for statistical significance.

RESULTS

In total, n=90 patients participated, of which n=86 provided data on HR-QoL. The 4 patients without HR-QoL data were either insufficient in their written language proficiency or did not respond to the distributed HR-QoL questionnaires. Descriptive information of patients is shown in Table 1.

Table 1. Patient characteristics of the total cohort and of the subgroups with and without VIPN using Common Toxicity Criteria of Adverse Events

	Total group (n=86)	No VIPN (n=19)	VIPN (n=67)	P-value
Age at diagnosis in years, mean (SD)	9.09 (5.12)	7.92 (5.51)	9.42 (5.00)	0.42
Sex, n (%)				0.40
Female	39 (45)	7 (37)	35 (52)	
Male	47 (55)	12 (63)	32 (48)	
Diagnosis, n (%)				0.12
ALL	56 (65)	10 (53)	46 (69)	
Hodgkin	16 (19)	3 (16)	13 (19)	
Other	14 (16)	6 (32)	8 (12)	
Ethnicity, n (%)				0.83
Caucasian	71 (83)	16 (84)	55 (82)	
Other	15 (17)	3 (16)	12 (18)	
Use of analgesics, n (%)				0.01
No	68 (79)	19 (100)	49 (73)	
Yes	18 (21)	0 (0)	18 (27)	
Time since diagnosis in days, mean (SD)	158 (145)	174 (148)	139 (141)	0.26
Mean cumulative VCR dose in mg per m^2 (SD)	14.8 (9.82)	13.12 (10.95)	15.23 (9.54)	0.07

VIPN: vincristine induced peripheral neuropathy, SD: standard deviation; ALL: acute lymphoblastic leukemia, VCR: vincristine

Time since diagnosis, sex, and disease were significantly associated with VIPN outcomes and total PedsQL scores (see Supplemental Table S1 and Supplemental Table S2) and were included, together with randomization status, in our multivariable analyses. In contrast, cumulative dose of VCR per m^2, use of analgesics, age and ethnicity were not significantly associated with VIPN outcomes (Supplemental Table S1). However, use of analgesics was significantly associated with the outcomes of the Pain domain of the PedsQL Cancer scores and therefore included in the multivariable model of this domain.

In total, n=41 (48%) developed VIPN according to CTCAE and n=52 (61%) according to ped-mTNS. In total, n=67 (78%) patients met criteria for VIPN according to ped-mTNS or CTCAE. The ped-mTNS cohort consisted of in total n=61 children (i.e. ≥5 years). Of n=61 children, n=26 (43%) developed VIPN according to both CTCAE and ped-mTNS.

Table 2. Differences in parent-reported HR-QoL between children with and without VIPN

	Generic Total score		Generic PSHS		Generic Physical functioning		Generic Emotional functioning		Generic Social functioning		Generic School functioning		Cancer Pain	
	Beta (95% CI)	P value	Beta (95% CI)	P value	Beta (95% CI)	P value	Beta (95% CI)	P value	Beta (95% CI)	P value	Beta (95% CI)	P value	Beta (95% CI)	P value
VIPN according to														
CTCAE or ped-mTNS	-8.96 (-14.48 to -3.43)	0.002	-5.95 (-11.24 to -0.67)	0.03	-13.44 (-19.48 to -7.41)	<0.001	-4.61 (-9.08 to -0.15)	0.04	-3.78 (-8.65 to 1.10)	0.13	-4.24 (-13.09 to 4.61)	0.35	-9.94 (-16.44 to -3.45)	0.003
CTCAE	-9.57 (-17.06 to -2.08)	0.01	-7.01 (-14.11 to 0.08)	0.05	-13.31 (-20.52 to -6.11)	<0.001	-7.68 (-12.84 to -2.52)	0.004	-3.36 (-9.11 to 2.40)	0.25	-6.82 (-18.56 to 4.92)	0.25	-11.70 (-19.22 to -4.19)	0.002
ped-mTNS	-9.96 (-15.83 to -4.08)	0.001	-6.87 (-12.38 to -1.37)	0.02	-12.71 (-20.19 to -5.2)	0.001	-1.63 (-7.37 to 4.11)	0.57	-6.98 (-12.63 to -1.34)	0.02	-6.65 (-15.34 to 2.03)	0.13	-12.79 (-21.62 to -3.95)	0.005
Severe VIPN according to														
CTCAE or ped-mTNS	-9.54 (-15.66 to -3.41)	0.003	-7.87 (-13.66 to -2.09)	0.008	-13.37 (-20.66 to -6.08)	<0.001	-7.41 (-12.76 to -2.06)	0.007	-6.86 (-12.56 to -1.16)	0.02	-8.22 (-17.94 to 1.50)	0.10	-15.27 (-22.73 to -7.82)	<0.001
CTCAE	-17.43 (-38.35 to 3.49)	0.10	-18.24 (-37.89 to 1.40)	0.07	-12.95 (-30.16 to 4.26)	0.14	-8.34 (-20.61 to 3.92)	0.18	11.16 (-2.97 to 25.29)	0.12	-16.00 (-46.95 to 14.94)	0.31	-13.21 (-29.35 to -2.93)	0.02
ped-mTNS	-10.02 (-15.83 to -4.22)	0.001	-8.11 (-13.45 to -2.77)	0.004	-13.49 (-21.11 to -5.87)	0.001	-6.14 (-11.89 to -0.40)	0.04	-9.07 (-14.67 to -3.47)	0.002	-10.86 (-19.19 to -2.52)	0.01	-16.99 (-25.60 to -8.37)	<0.001

PSHS: psychosocial health summary score, CI: confidence Interval, VIPN: vincristine induced peripheral neuropathy, CTCAE: common terminology criteria of adverse events, ped-mTNS: pediatric modified Total Neuropathy Score. Results represents outcomes of a multivariable model, in which outcomes are additionally corrected for randomization of administration duration (push versus one-hour infusions), time since diagnosis, sex, diagnosis. The Cancer Pain scale was additionally corrected for use of analgesics. No VIPN was used as reference category in all analyses

Table 3. Differences in self-reported HR-QoL between children with and without VIPN

	Generic Total score		Generic PSHS		Generic Physical functioning		Generic Emotional functioning		Generic Social functioning		Generic School functioning		Cancer Pain	
	Beta (95% CI)	P value	Beta (95% CI)	P value	Beta (95% CI)	P value	Beta (95% CI)	P value	Beta (95% CI)	P value	Beta (95% CI)	P value	Beta (95% CI)	P value
VIPN according to														
CTCAE or ped-mTNS	-8.38 (-13.76 to -3.00)	0.003	-3.38 (-8.37 to 1.62)	0.18	-17.19 (-24.45 to -9.92)	<0.001	-8.02 (-14.11 to -1.93)	0.01	-4.32 (-9.47 to 0.83)	0.10	0.56 (-7.34 to 8.45)	0.89	-19.15 (-26.82 to -11.48)	<0.001
CTCAE	-6.91 (-14.29 to 0.47)	0.07	-4.23 (-10.90 to 2.43)	0.21	-9.58 (-19.32 to 0.16)	0.05	-6.81 (-14.64 to 1.03)	0.09	-4.85 (-11.25 to 1.55)	0.14	-2.93 (-13.53 to 7.67)	0.59	-25.28 (-34.85 to -15.72)	<0.001
ped-mTNS	-11.42 (-16.87 to -5.97)	<0.001	-6.46 (-11.57 to -1.35)	0.01	-19.75 (-27.31 to -12.19)	<0.001	-9.05 (-15.57 to -2.53)	0.007	-6.90 (-12.16 to -1.65)	0.01	-4.01 (-12.26 to 4.24)	0.34	-17.98 (-26.04 to -9.56)	<0.001
Severe VIPN according to														
CTCAE or ped-mTNS	-10.98 (-16.41 to -5.55)	<0.001	-7.22 (-12.27 to -2.17)	0.006	-17.14 (-24.62 to -9.66)	<0.001	-10.32 (-16.52 to -4.11)	0.001	-5.50 (-10.73 to -0.26)	0.04	-8.65 (-16.69 to -0.60)	0.04	-18.41 (-26.61 to -10.20)	<0.001
CTCAE	-12.41 (-33.30 to 8.48)	0.24	-9.08 (-27.76 to 9.60)	0.34	-18.14 (-40.92 to 4.65)	0.12	-12.54 (-30.76 to 5.68)	0.18	-11.64 (-26.20 to 2.91)	0.12	-20.69 (-49.81 to 8.43)	0.16	-25.19 (-53.32 to 2.93)	0.08
ped-mTNS	-12.24 (-17.63 to -6.84)	<0.001	-8.43 (-13.44 to -3.41)	0.001	-18.60 (-26.23 to -10.98)	<0.001	-10.34 (-16.82 to -3.86)	0.002	-7.19 (-12.42 to -1.96)	0.007	-10.81 (-18.76 to -2.87)	0.008	-19.29 (-27.64 to -10.95)	<0.001

PSHS: psychosocial health summary score, CI: confidence Interval, VIPN: vincristine induced peripheral neuropathy, CTCAE: common terminology criteria of adverse events, ped-mTNS: pediatric modified Total Neuropathy Score. Results represents outcomes of a multivariable model, in which outcomes are additionally corrected for randomization of administration duration (push versus one-hour infusions), time since diagnosis, sex, diagnosis. The Cancer Pain scale was additionally corrected for use of analgesics. No VIPN was used as reference category in all analyses.

In total, n=42 (49%) patients developed severe VIPN according to either CTCAE (n=8 (9%)), ped-mTNS (n=38 out of n=61 (62%)) or both (n=4 (7%)) at any time point during the first year of treatment. In Supplemental Table S3 the HR-QoL scores per time point are reported.

Relation between PedsQL generic self and proxy assessments and VIPN

Overall, estimated PedsQL Generic Total score was 84.57 (parent-reported) and 85.16 (self-reported). The timing of VIPN and HR-QoL assessments in relationship to VCR treatments are presented in Supplemental Figure S3.

Results of the uncorrected association between VIPN and PedsQL scores are presented in Supplementary Tables S4 and S5.

There was a significantly lower HR-QoL (proxy and self-assessments) in children with VIPN compared to children without VIPN for both PedsQL Generic total score as well as all sub domains except School Functioning (Tables 2 and 3; Figure 1). Interestingly, in children with severe VIPN according to the ped-mTNS, there was a significantly lower score on (proxy- and self-reported) school functioning compared to children without VIPN.

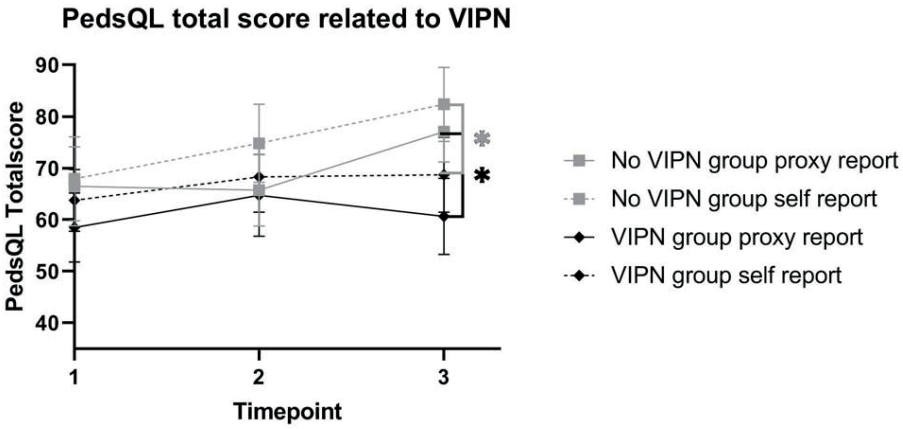

Figure 1. Total PedsQL generic score divided in pediatric patients with and without vincristine-induced peripheral neuropathy according to either the Common Terminology Criteria for Adverse Events or pediatric modified Total Neuropathy Score

**represents p values < 0.05 per timepoint between the groups with and without VIPN, VIPN: vincristine induced peripheral neuropathy, PedsQL: Pediatric Quality of Life Inventory*

Relation between PedsQL Cancer self and proxy assessments and VIPN

Results of the uncorrected analyses and of the univariable association with covariates are presented in Supplementary Tables S4 and S5. Estimated PedsQL Cancer domain Pain and Hurt score was 85.28 (parent-reported) and 97.57 (self-reported). Similar to the PedsQL Generic outcomes, these and other outcomes of PedsQL Cancer Pain and Hurt scores according to VIPN status per time point of other domains are presented in Supplementary Figure S1. HR-QoL according to PedsQL Cancer Pain and Hurt in proxy as well as self-assessments was significantly lower in our multivariable model in patients with VIPN compared to children without VIPN, irrespective of VIPN measurement tool (Table 2 and 3). In patients with severe VIPN measured by ped-mTNS there was a significant lower HR-QoL compared to children without severe VIPN. However, the results of self-assessment were not statistically significant when only the CTCAE was taken into consideration, but the estimated difference in PedsQL score in children with and without VIPN according to the CTCAE (β) was -25.19 (Tables 2 and 3; Figure 2). PedsQL Cancer scores per time point, subdivided according to VIPN status, are presented in Figure 2.

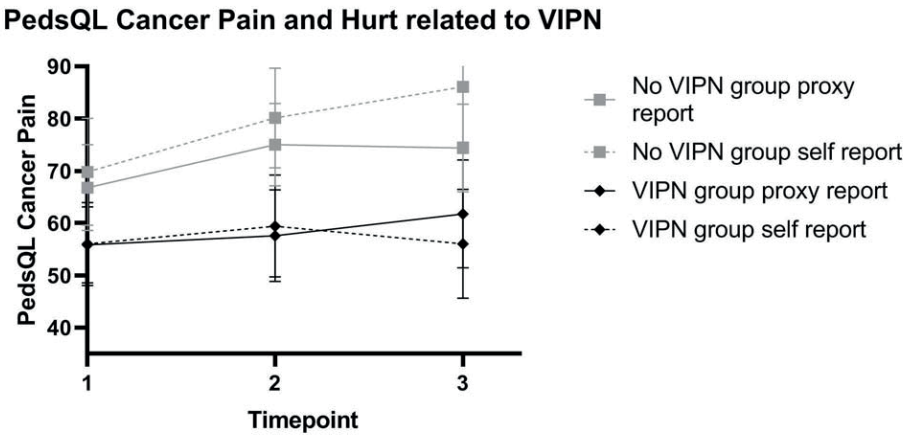

Figure 2. Total PedsQL Cancer scores Pain and Hurt domain divided in children with and without vincristine-induced peripheral neuropathy according to the Common Terminology Criteria for Adverse Events or pediatric modified Total Neuropathy Score

All results at different time points were significant with a p<0.05 VIPN: vincristine induced peripheral neuropathy, PedsQL: Pediatric Quality of Life Inventory

DISCUSSION

This study shows that pediatric oncology patients with VIPN have a significantly reduced HR-QoL compared to patients without VIPN. This finding is consistent, as it was found irrespective of method of assessment of VIPN (CTCAE or ped-mTNS), and both in self- as well as in proxy-assessments, and on multiple HR-QoL domains.

VCR is an important part of many pediatric oncology treatment protocols, but VIPN is a severe toxicity with symptoms that can last long after treatment cessation[37,38]. Even though VCR has been used for decades in children and its association with VIPN is well-known, as far as we know, this trial is the first assessing the consequences of VIPN on HR-QoL in children currently treated for childhood cancer. However, the effect of VIPN on HR-QoL in pediatric oncology *survivors* was previously reported. The study of Tay et al.[38] investigated 101 survivors of childhood ALL (mean time post-treatment: 4.11 years) and reported a significant association between VIPN and the PedsQL, with reduced scores on the generic domains Physical Functioning and Social functioning in patients with VIPN.

Ultimately, this study shows the necessity for improving VCR treatment in children with cancer as a way to improve overall HR-QoL of patients during oncologic treatment. Therefore, dosing of VCR should be done on a more individualized basis. For instance, it was demonstrated that certain genetic aberrations, like a single nucleotide polymorphism in centrosomal protein 72 (CEP72) influence the development of VIPN in children[39]. Following this, studies could be undertaken that use these genetic aberrations to dose modify VCR in certain populations requiring more or less. Another approach would be to study pharmacokinetics of VCR. There is no established target VCR exposure yet, but reliable VCR PK data have been collected that could contribute to this[13,40]. When the target VCR exposure is known, VCR doses could be adapted accordingly. Finally, improving administration duration of VCR can optimize VCR treatment[16,40,41]. When one of these, or other, approaches are studied and used for the optimization of VCR treatment, ideally the effect of the intervention on HR-QoL of children should also be studied.

Finally, HR-QoL of children is dependent on the coping and adjustment possibilities of the child and the surrounding family. Poverty and social isolation contribute to ongoing and/or escalation of distress, similar to other (pre-existing) stressors[42]. In general, it was demonstrated that all factors that are associated with health disparities in pediatric oncology were related to both wellbeing and overall adjustment. Also family structure and beliefs and the natural coping ability to function are related to

the adaptive adjustment to cancer (treatment). Therefore, it is important to screen for these factors in families[43]. Interventions aimed to improve HR-QoL in pediatric oncology patients should also take these psychosocial factors into account besides solely focusing on physical functioning.

The strengths of this trial, among others, are the comprehensive and prospective measurements of VIPN. For this study two instruments that include physical examination were used to assess VIPN at several occasions during treatment. These assessments were done using specifically trained physical therapists at all study sites. Currently, ped-mTNS is recommended for VIPN assessment in children, as shown in a systematic review[44]. In other studies, VIPN assessment was frequently done only by retrospective CTCAE assessment[13]. Overall, the effects of VIPN on HR-QoL seem to be stronger and more significant when VIPN is measured by ped-mTNS. Previous literature showed that ped-mTNS assessment is more sensitive in detecting VIPN than CTCAE assessment[44], which is supported by the fact that in our study more children with VIPN were identified by ped-mTNS than by CTCAE. This could be an explanation for the enhanced effect of VIPN on HR-QoL, when this was assessed by ped-mTNS. Our data also shows that the negative effect of VIPN on HR-QoL is stronger when VIPN is severe, even though the results of severe VIPN did not reach statistical significance for the CTCAE outcomes. This lack of significance is likely due to the low patient numbers with severe VIPN according to the CTCAE (n=8).

Our study had some limitations. First of all, our results rely partly on HR-QoL assessment by proxy responders. It was previously shown in healthy children that there is a relation between the mental health status of a parent and the proxy ratings they score[45]. Secondly, this study reports on children with different disease types and treatment phases of pediatric cancer. Treatment of different diseases and treatment phases are not uniform regarding the administered VCR doses. Even though we corrected our results for disease type and did not find an association between cumulative VCR dose per m^2 in the relation between VIPN and HR-QoL, differences per treatment phase could still be of importance, also due to the effect of other (chemotherapeutic) treatment modalities. De Vries et al.[11] have shown for instance that dexamethasone treatment, which is frequently used in some treatment phases of ALL but less frequent in treatment protocols of other diseases, is significantly associated with worse HR-QoL in children with cancer. Therefore, it would be useful to investigate the VIPN/HR-QoL relation in a more uniform patient population, to see if this association might be more attenuated in some patients. Moreover, the measurements of VIPN were assessed by a clinician, whereas HR-QoL was assessed by patients or proxies. This could have resulted in different perceptions regarding the occurrence of VIPN between clinicians

and patients (for example: clinicians that identify VIPN based on loss of deep tendon reflexes and mild constipation and patients not reporting VIPN due to lack of sensory or motor symptoms). However, VIPN is most adequately assessed using a combination of questions and physical examination. Therefore, it would be suboptimal to use questionnaires only for assessing VIPN. This is vice versa for HR-QoL, which is most adequately assessed using standardized questionnaires such as the PedsQL, which rely on the assessment by patients and proxies and not by clinicians. Also, in the PedsQL Cancer module Pain and Hurt, the reported painful symptoms are not necessarily referring to VIPN, but other causes of pain as well. Finally, the number of included patients in this cohort was relatively low which has limited the amount of possible confounders that could be included in the corrected analysis. With a larger sample size, the influence of other confounders such as the use of additional neurotoxic agents or the use of central nervous system directed therapy could be included in the analyses as well.

In conclusion, this study shows that over time VIPN is negatively associated with HR-QoL in children currently treated for a malignancy. These findings are robust, irrespective of self- or proxy assessment and VIPN assessment method (CTCAE or pedmTNS). Even though VCR is already used for decades and in multiple diseases, these findings underline the necessity for new studies aimed at optimizing VCR treatment using interventions directed at reducing the incidence and severity of VIPN during treatment of childhood cancer. Individualized treatment of VCR, for instance by using pharmacokinetic and pharmacogenomic data, will result in a tailored exposure to VCR resulting in treatment with maximum efficacy, reduced VIPN and thereby increased HR-QoL in pediatric oncology patients.

SUPPLEMENTAL MATERIALS

Supplemental Table S1. Association between parent-proxy reported PedsQL and vincristine induced peripheral neuropathy and effect of covariates

Supplemental Table S2. The association between pedsQL outcomes and randomization (VCR administrations given as either push-injection or one-hour infusion)

Supplemental Table S3. Mean Generic and Cancer PedsQL scores of proxy- and self-report per time point

Supplemental Table S4. Univariate differences in parent-reported HR-QoL between children with and without VIPN

Supplemental Table S5. Univariate differences in self-reported HR-QoL between children with and without VIPN

Supplemental Figure S1. PedsQL scores per domain related to VIPN

Supplemental Figure S2. Overview of measurements per disease

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Vincristine-Induced Peripheral Neuropathy in Pediatric Oncology: A Randomized Controlled Trial Comparing Push Injections with One-Hour Infusions (The VINCA Trial)

Mirjam Esther van de Velde, Gertjan J.L. Kaspers, Floor C.H. Abbink, Jos W.R. Twisk, Inge M. van der Sluis, Cor van den Bos, Marry M. van den Heuvel–Eibrink, Heidi Segers, Christophe Chantrain, Jutte van der Werff Ten Bosch, Leen Willems and Marleen H. van den Berg

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ABSTRACT

Vincristine (VCR) is a frequently used chemotherapeutic agent. However, it can lead to VCR-induced peripheral neuropathy (VIPN). In this study we investigated if one-hour infusions of VCR instead of push-injections reduces VIPN in pediatric oncology patients. We conducted a multicenter randomized controlled trial in which participants received all VCR administrations through push injections or one-hour infusions. VIPN was measured at baseline and 1–5 times during treatment using Common Terminology Criteria of Adverse Events (CTCAE) and pediatric-modified Total Neuropathy Score. Moreover, data on co-medication, such as azole antifungals, were collected. Overall, results showed no effect of administration duration on total CTCAE score or ped-mTNS score. However, total CTCAE score was significantly lower in patients receiving one-hour infusions concurrently treated with azole antifungal therapy (β = -1.58; p = 0.04). In conclusion, generally VCR administration through one-hour infusions does not result in less VIPN compared to VCR push injections in pediatric oncology patients. However, one-hour infusions lead to less severe VIPN compared to push-injections when azole therapy is administered concurrently with VCR. These results indicate that in children treated with VCR and requiring concurrent azole therapy, one-hour infusions might be beneficial over push injections, although larger trials are needed to confirm this association.

INTRODUCTION

Vincristine (VCR) is a frequently-used chemotherapeutic agent in pediatric oncology since many decades[1,2]. VCR inhibits the mitotic spindle in the cell, thereby blocking cell division[3,4]. In the liver, it is metabolized into M1 by the cytochrome P450 (CYP) 3A enzymes[5]. A major adverse effect of VCR is neurotoxicity, which is characterized by autonomic and peripheral sensory-motor neuropathy and reported in 12–87% of VCR-exposed children[1,6-8]. Symptoms of VCR-induced peripheral neuropathy (VIPN) include paresthesia, muscle weakness, areflexia, pain, and diminished sensibility[9-12]. It usually starts after a few administrations and symptoms often reside several months after treatment cessation[1,2]. VIPN can lead to suboptimal treatment due to dose reductions or omissions of VCR[10,13]. VIPN is dose-dependent, with single administration doses exceeding 2.0 mg/m^2 leading to intolerable VIPN in children[14]. Children of older age or Caucasian ancestry seem more vulnerable. Furthermore, genetic predispositions and pharmacokinetics (PK) of VCR influence VIPN development[1,6,14-21]. Moreover, multiple studies have shown that concurrent azole antifungal and VCR treatment leads to more and severe VIPN in children, due to competitive interaction by the CYP enzyme[22-24]. This is clinically relevant, since azole antifungals are frequently used to prevent or treat invasive fungal infections in pediatric cancer[25]. Although VIPN is dose-limiting[1,14], prolongation of VCR administration showed that the single administration dose can be increased without leading to intolerable VIPN in children. In two studies using continuous VCR infusion up to five days, cumulative VCR doses of 4.0 mg/m^2 were well tolerated[26,27]. This could be due to lower peak-plasma concentrations that are related to longer lasting infusions, which seem associated with less VIPN[28]. Yet, multiple day VCR infusions are costly and cumbersome. Therefore, in clinical practice VCR is usually administered intravenously (iv) through a short-term push injection or infusion (up to 15 min). Sometimes it is administered through a one-hour infusion. Both push and one-hour administrations use standardized VCR doses of 1.5–2.0 mg/m^2 (maximum 2.0 mg)[29]. However, the effect of prolonging VCR infusion on VIPN using standardized dosing regimens, is unknown. Therefore, we conducted a randomized controlled trial (RCT) to determine whether intravenous one-hour infusions of VCR are associated with less VIPN compared to intravenous push injections in pediatric oncology patients. In addition, we evaluated the potentially modifying effect of co-medication (i.e., concurrent azole antifungal treatment) on this association.

MATERIALS AND METHODS

Trial Design

This study is an international, multi-center, randomized controlled trial. In total, ten pediatric oncology treatment centers across the Netherlands (four) and Belgium (six) participated in the study. Written informed consent was obtained from parents or guardians of all study participants aged 2–17 years and their children (12–17 years). The protocol and consent form were approved by the Institutional Review Board (IRB) of Amsterdam UMC, location VUmc (IRB number: 2014–268, EUDRACT number: 2014-001561-27).

The study was conducted in accordance with the Declaration of Helsinki. An independent safety committee was updated annually on study progress, incidence and nature of serious adverse events occurring during the study. This committee did not find any reason to discontinue the study.

Study Participants

The study population consisted of newly diagnosed pediatric oncology patients aged 2–18 years. Patients were eligible for study participation when their treatment protocol included at least four VCR administrations within six weeks. Therefore, patients with the following diagnosis and corresponding treatment protocols could be included: acute lymphoblastic leukemia (ALL) (DCOG ALL-11 protocol[29], EsPhALL protocol[30], or EORTC-58081-CLG guideline[31]), Hodgkin's lymphoma (EuroNet-PHL-C1 protocol[32] or C2 protocol[33]), nephroblastoma (SIOP Wilms 2001 protocol[34]), and rhabdomyosarcoma (EpSSG RMS 2005 protocol[35]). Furthermore, patients with low-grade glioma (LGG) (SIOP-LGG 2004[36] protocol) and medulloblastoma (ACNS0331[37] protocol or ACNS0332[38] protocol) were eligible if their presenting symptoms did not include any sensory or motor symptoms of their limbs. Patients with mental retardation or pre-existent peripheral neuropathy were excluded from participation.

Trial Regimen

Participating patients underwent baseline VIPN assessment to exclude pre-existing peripheral neuropathy. Then, patients were 1:1 randomized using Tenalea software (Trans European Network for Clinical Trials Services) to receive all VCR administrations through push injections or one-hour infusions. To ensure equal distribution, stratified block randomization (maximum block size: four) was used according to age group (2–10 years or 11–18 years), gender, and country. During the first year of treatment, VIPN was measured 1–3 times, depending on protocol treatment duration and number of VCR administrations (see Supplementary Figure S1 for the detailed

measurement schedules). In *n* = 5 patients, five measurements were done within the first year as these patient were treated according to a rather dense protocol (i.e. many VCR administrations within a relatively short period of time).

VCR was administered using a standard dose of 1.5 mg/m^2 or 2 mg/m^2 (maximum 2 mg), depending on treatment protocol. A push injection was administered in an injection of 10 mL 0.9% NaCl or iv bag of 50 mL 0.9% NaCl. Total administration time of a push injection was 1–5 min. One-hour infusions were administered using an iv bag of 50 mL of NaCl 0.9%.

Assessments of VIPN and Other Study Outcomes

The primary endpoint of the current study was VIPN measured by the CTCAE (version 4.03[39]) (CTCAE). The items peripheral sensory neuropathy (range 0–5), peripheral motor neuropathy (range 0–5), constipation (range 0–5), and neuralgia (range 0–3) were assessed. A CTCAE score of ≥2 on at least one of the four items was defined as VIPN and ≥3 or higher as severe VIPN. Moreover, all four items were summed into a total CTCAE score (maximum 18).

Secondary endpoints of the current study included VIPN measured by the Dutch version of the ped-mTNS[40-42] and by the use of specific analgesics for neuropathic pain. The ped-mTNS is a validated instrument, including a questionnaire (sensory, functional and autonomic symptoms) and physical examination, developed to assess VIPN in children aged ≥ 5 years. Therefore, this instrument was not used in children aged < 5 years. A total ped-mTNS score of ≥5 was defined as VIPN (total scoring range of ped-mTNS: 0–32)[40]. All VIPN measurements were performed in each hospital by specifically trained physicians or physical therapists during regular hospital visits of the patient.

Data on co-medication were collected by chart-based review. Amitriptyline and gabapentin are frequently used for the treatment of neuropathic pain caused by VIPN[6]. Therefore, children using these analgesics for ≥14 days were considered to have VIPN. Furthermore, data regarding concurrent azole antifungal treatment were collected. Patients were considered to have been treated with this concurrent therapy when azoles were used during the week preceding or following VCR administration and when ≥50% of VCR administrations between two succeeding study measurements were given with concurrent azole therapy.

This paper reports on data collected during the first year of treatment.

Sample Size Calculation

Initial sample size calculation was based on total CTCAE scores (primary endpoint) using data of two studies[15,41] in which a mean maximum (standard deviation (SD)) CTCAE score of 2.43 (1.07) was reported. However, since our study population included children with different pediatric oncology diagnoses, we increased the expected SD to 1.3 instead of 1.07. A difference in CTCAE score of at least 1.0 was considered to be clinically relevant. This resulted in a targeted sample size of at least 70 patients (35 patients in each intervention group; α = 5%, power = 90%). To compensate for an expected drop-out rate of 25%, we aimed to include 44 patients in each group (total: 88).

Statistical Analyses

Descriptive data of normally and skewed distributed variables were reported as means (SD) and medians (interquartile range (IQR)), respectively. Possible associations between VCR administration method (push injection or one hour infusion) and VIPN were evaluated in multiple ways. Mean, median scores and proportions were compared using *t*-tests, Mann-Whitney U and chi-square tests, respectively. Moreover, linear mixed effects model analyses were used to assess the association between administration method and total CTCAE score as well as total ped-mTNS score, thereby correcting for the correlated observations within and between patients. Logistic generalized estimating equations (GEE) analyses were used for dichotomized CTCAE and ped-mTNS scores (i.e., having VIPN yes or no) and use of analgesics. Furthermore, the possible confounding and/or modifying effect of the covariates age, sex, cumulative VCR dose per m^2, cancer diagnosis, concurrent azole antifungal treatment (yes/no), and self-declared ancestry was studied. When a modifying effect on the association between randomization and VIPN for one of the covariates was found (i.e., *p* value of the interaction term <0.05), results were reported for each category of this covariate separately.

All participants were included in our intention-to-treat analyses. A two-tailed *p*-value of <0.05 was considered statistically significant, analyses were performed using SPSS 26.0 (Chicago, USA) software.

RESULTS

Participants

From September 2014 through January 2018, a total of 90 participants (n = 45 push administration, n = 45 one-hour administration) were enrolled (Figure 1). In general, baseline characteristics of the two groups were well-balanced (Table 1). Most participants were treated for acute lymphoblastic leukemia (ALL). Data of all 90 randomized patients were used for analysis. There was no difference in relapse rate ($p > 0.99$) or mortality ($p = 0.62$) between the two groups. In total, eight patients (9%) dropped-out during the trial, which was less than anticipated. Results of baseline peripheral neuropathy scores are reported in Supplementary Table S1.

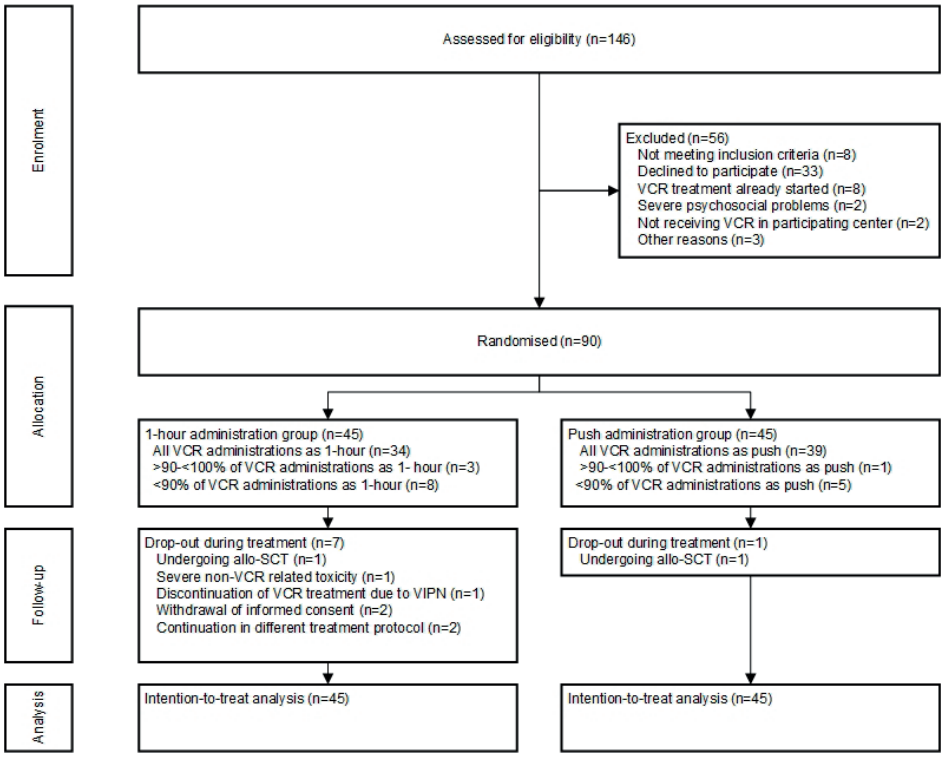

Figure 1. Flow diagram of screening, randomization, and follow-up.
VCR: vincristine; allo-SCT: allogeneic stem-cell transplantation.

Table 1. Demographic and clinical characteristics of the participants at baseline.

	One-Hour Administration Group (*n* = 45)	Push Administration Group (*n* = 45)
Sex		
Male	26 (58)	24 (53)
Female	19 (42)	21 (47)
Age, years (mean (SD))	9.06 (5.11)	9.29 (5.25)
Age ≥ 5 years and included for ped-mTNS assessment	32 (71)	34 (76)
Disease		
Acute lymphoblastic leukemia	29 (64)	29 (64)
Hodgkin lymphoma	7 (16)	11 (24)
Nephroblastoma	6 (13)	2 (4)
Medulloblastoma	1 (2)	1 (2)
Rhabdomyosarcoma	2 (4)	0 (0)
Low-grade glioma	0 (0)	2 (4)
Ancestry [a]		
Europe	36 (80)	37 (82)
Eastern Asia	1 (2)	0 (0)
Latin-America (including Caribbean)	1 (2)	2 (4)
Middle-East (including Northern Africa)	3 (7)	3 (7)
Sub-Saharan Africa	1 (2)	0 (0)
Combination	2 (4)	3 (7)
Missing	1 (2)	0 (0)
No. (%) of patient needing VCR dose reductions or omissions	1 (3)	0 (0)
No. (%) of patients using analgesics for neuropathic pain	9 (20)	11 (24)
Relapse (No. (%))	2 (4)	1 (2)
Death (No. (%))	3 (7)	1 (2)

Values represent the number (%) of participants, unless indicated otherwise. SD: standard deviation; ped-mTNS: pediatric modified Total Neuropathy Score; VCR: vincristine; [a]: Ancestry was self- or parent-reported; participants could only be included in one category.

Primary Endpoints

Overall, 43 out of 90 children developed VIPN as measured by Common Terminology Criteria of Adverse Events (CTCAE), of whom 9 had severe VIPN. Furthermore, according to three out of four CTCAE items more children in the push group were identified with VIPN compared to the one-hour group, although these differences were not statistically significant. Finally, 55 out of in total *n* = 66 patients aged ≥5 years developed VIPN based on pediatric modified Total Neuropathy Score (ped-mTNS) assessment of children ≥5 years of age. Results are summarized in Table 2. No statistically significant difference in VIPN as measured by the total CTCAE score, ped-mTNS score or use of analgesics between patients in both randomization groups was found (Figure 2 and Table 3).

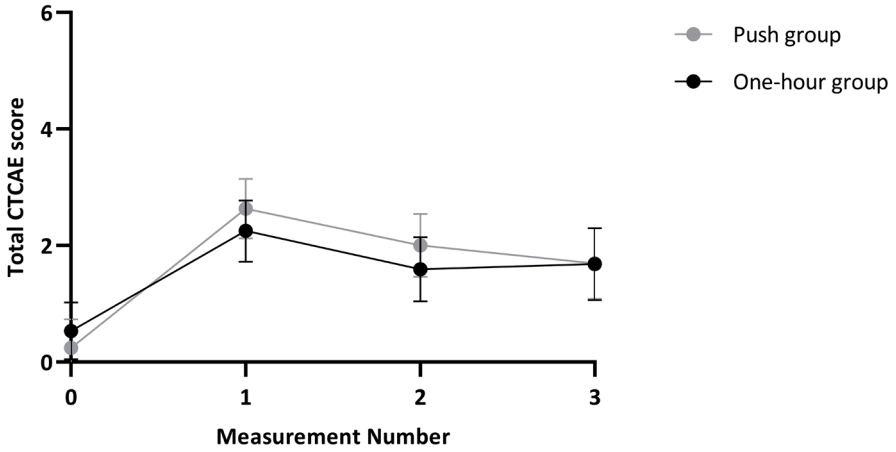

Figure 2. Total Common Terminology Criteria of Adverse Events score per administration method. CTCAE: Common Terminology Criteria of Adverse Events, dots represent the estimated values and lines of the 95% confidence interval of this estimate. Numbers per measurement: t = 0: n = 90, t = 1: n = 80, t = 2: n = 72, t = 3: 58. Although for n = 5 patients five measurements were available, results of the latter two of these measurements were not taken into account for this graph due to the small number of patients.

Since concurrent azole treatment appeared to be an effect modifier (interaction term: p = 0.03), results are reported separately for measurements with (n = 19 patients) and without (n = 71 patients) concurrent azole treatment (Table 3 and Figure 3). Within the group of measurements with concurrent azole antifungal treatment (one-hour group: n = 10, push group: n = 9), total CTCAE scores were significantly lower in the one-hour group compared to the push group. Our results did not meaningfully change after adjustment for several covariates or when dichotomized outcomes were used (Supplementary Table S2).

Secondary Endpoints

Analyses regarding the ped-mTNS provided similar results as those of CTCAE. Overall, no statistically significant effect of administration method on total ped-mTNS score was found. Again, when results were separately analyzed for measurements with concurrent azole treatment, there was a trend towards higher ped-mTNS scores in the push-administration group (Table 3). Results were comparable when additionally corrected or when using dichotomized ped-mTNS scores (Table S2). The risk of having VIPN measured by the use of analgesics was not significantly different for participants in the two randomization groups as well as within the two azole subgroups (Table 3 and Table S2).

Table 2. Incidence of vincristine induced peripheral neuropathy of participants in both randomization groups.

	One-Hour (*n* = 45) *n* (%) *	**Push (*n* = 45) *n* (%) ***	***p* Value**
VIPN based on CTCAE	21 (46.7)	23 (51.1)	0.67
Severe VIPN based on CTCAE	3 (6.7)	6 (13.3)	0.49
VIPN based on ped-mTNS	27 (84.4) **	28 (82.4) **	0.83
VIPN pain medication	9 (20.0)	11 (24.4)	0.61
VIPN based on CTCAE item constipation	3 (6.7)	8 (17.8)	0.20
VIPN based on CTCAE item peripheral sensory neuropathy	5 (11.1)	6 (13.3)	0.75
VIPN based on CTCAE item peripheral motor neuropathy	17 (37.8)	15 (33.3)	0.66
VIPN based on CTCAE item neuralgia	6 (13.3)	12 (26.7)	0.11
CTCAE score (median [IQR])	2.00 [1.00–3.00]	1.50 [0.00–3.00]	0.43
ped-mTNS score (median [IQR])	6.00 [3.75–10.00] **	5.50 [2.00–9.00] **	0.19
VIPN outcomes without concurrent azole antifungals	**One-hour (*n* = 35)**	**Push (*n* = 36)**	
CTCAE score without concurrent azole treatment (median [IQR])	2.00 [1.00–2.75]	1.00 [0.00–3.00]	0.20
ped-mTNS score without concurrent azole treatment (median [IQR])	5.50 [3.25–9.00] ***	5.00 [2.00–8.00] ***	0.09
VIPN outcomes with concurrent azole antifungals	**One-hour (*n* = 10)**	**Push (*n* = 9)**	
CTCAE score with concurrent azole treatment (median [IQR])	2.00 [1.00–3.00]	3.00 [1.00–6.00]	0.21
ped-mTNS score with concurrent azole treatment (median [IQR])	8.50 [3.75–11.50] ****	10.00 [7.00–13.00] ****	0.39

VIPN: vincristine induced peripheral neuropathy, CTCAE: common terminology criteria of adverse events, ped-mTNS: pediatric-modified Total Neuropathy Score, IQR: interquartile range * All scores indicate *n* (%) unless indicated otherwise ** Total group was *n* = 32 in the iv one-hour group and *n* = 34 in the iv push group since ped-mTNS assessment was only done in children aged ≥5 years, *** Total group was *n* = 23 in the iv one-hour group and *n* = 27 in the iv push group, **** Total group was *n* = 9 in the iv one-hour group and *n* = 7 in the iv push group.

Table 3. The effect of VCR administration method (push administration versus one-hour administration) on vincristine-induced peripheral neuropathy.

	Total Group (*n* = 90)		Subgroup of Participants without Concurrent Azole Treatment (*n* = 71)		Subgroup of Participants with Concurrent Azole Treatment (*n* = 19)	
	β/OR (95% CI)	*p*-Value	β/OR (95% CI)	*p*-Value	β/OR (95% CI)	*p*-Value
CTCAE [a]					–	
Total score during treatment	−0.29 (−0.89 to 0.31)	0.34	−0.12 (−0.73 to 0.49)	0.69	1.58 (−3.11 to −0.05)	0.04
Ped-mTNS [a]						
Total score during treatment	−0.25 (−1.95 to 1.45)	0.77	0.03 (−1.78 to 1.84)	0.97	−1.80 (−5.33 to 1.72)	0.31
Participants with VIPN [a,b]						
Based on CTCAE	1.21 (0.43 to 1.46)	0.45	0.93 (0.49 to 1.79)	0.84	0.26 (0.04 to 1.67)	0.16
Based on ped-mTNS	1.12 (0.58 to 2.16)	0.74	1.33 (0.63 to 2.80)	0.46	0.22 (0.02 to 2.76)	0.24
Based on analgesics use	0.76 (0.30 to 1.94)	0.57	0.67 (0.21 to 2.18)	0.51	0.86 (0.17 to 4.43)	0.86

OR: odds ratio; 95% CI: 95% confidence interval; Common Terminology Criteria of Adverse Events; ped-mTNS: pediatric modified Total Neuropathy Score; VIPN: vincristine-induced peripheral neuropathy; [a]: Push administration group served as reference group, [b] No VIPN group served as reference group. Results regarding CTCAE were the primary endpoint in this study, results regarding ped-mTNS and analgesics were secondary outcomes.

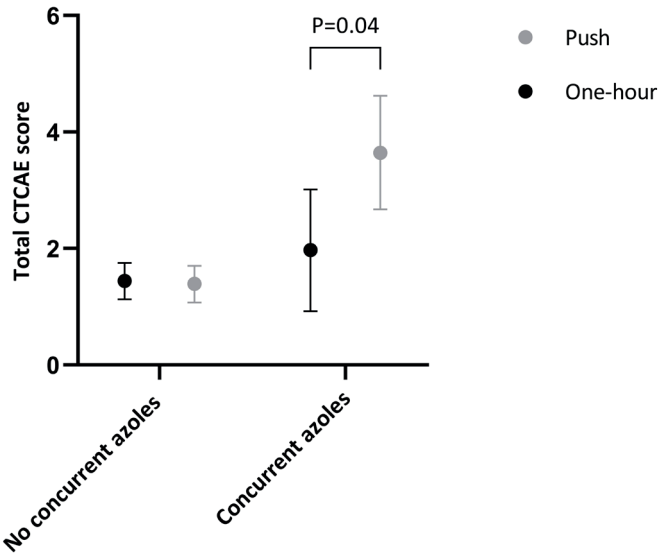

Figure 3. Total Common Terminology Criteria of Adverse Events score per randomization method and taking concurrent azole antifungal treatment into account. CTCAE: Common Terminology Criteria of Adverse Events, dots represent the estimated values and lines of the 95% confidence interval of this estimate. Numbers per group: no concurrent azoles: push group: *n* = 36, one-hour group: *n* = 40; concurrent azoles: push group: *n* = 9, one-hour group: *n* = 10.

DISCUSSION

In the current study, in which 90 pediatric oncology patients were randomized to receive VCR administrations through iv push injections or one-hour infusions, overall VIPN did not differ between the two groups. However, when VCR was administered concurrently with azole antifungals, children in the one-hour group had a significantly lower total CTCAE score than those in the push group. When VIPN was assessed by ped-mTNS scores or by dichotomized outcomes (having VIPN or not), no significant differences were found between push administrations and one-hour infusions, irrespective of concurrent azole treatment, although a trend in the same direction as CTCAE results was shown.

VIPN is a debilitating toxicity with symptoms still present in adult survivors of childhood cancer[43]. Therefore, the overarching goal of this trial was to study an intervention possibly resulting in reduced VIPN during treatment of childhood cancer. To the best of our knowledge, this is the first RCT studying the effect of administration duration of VCR on VIPN, either in children or adults. During this trial we evaluated VIPN prospectively and longitudinally with repeated measurements. At each hospital uniformly trained assessors evaluated VIPN using two different instruments (CTCAE and ped-mTNS) including standardized physical examination[40]. Especially the ped-mTNS has been systematically reviewed and is currently recommended for the assessment of VIPN in children[44].

Our results show that one-hour infusions result in lower total CTCAE scores when VCR is concurrently used with azole antifungals. In general, this concurrent treatment is associated with increased incidence and severity of VIPN[22-24]. In our study, estimated CTCAE score was 1.41 (95%CI: 1.19 to 1.64) for measurements without concurrent azole treatment and more than twice as high (2.87 (95%CI: 2.15 to 3.58)) for measurements with concurrent azole treatment ($p < 0.001$). However, when these scores of VIPN with concurrent azole antifungal use were evaluated for patients in each randomization group separately, estimated CTCAE score of patients in the one-hour group was 1.97 (95%CI: 0.92 to 3.01) vs. 3.64 (95%CI: 2.67 to 4.62) in the push group for measurements with concurrent azole treatment. These results show that concurrent treatment of azole antifungals and VCR has a smaller impact on VIPN when VCR is administered through one-hour infusions. This could also well be true for concurrent use with other strong CYP3A inhibitors, such as anti-retroviral drugs or carbamazepine.

Since concurrent use of azole antifungals and VCR are generally avoided, alternative treatment for invasive fungal infections (IFI) must be used, such as echinocandins

or (liposomal) amphotericin B. However, these agents have several disadvantages including high costs, iv administration only, and lack of evidence of superior efficacy over azole antifungals in children[45-47]. Therefore, in clinical practice IFI are frequently treated with azole antifungals irrespective of concurrent VCR treatment[48,49]. Although in clinical practice treatment with azole antifungals is sometimes discontinued 24 hours before VCR administration, this interval is too short, based on the half-life of azole antifungals, to have an impact on this drug-drug interaction[50]. It should be noted, that the absolute number of patients with concurrent VCR and azole treatment was small in our study (*n* = 19, 21%). Therefore, future studies should be undertaken to replicate our results and indisputably confirm the favorable effect of one-hour infusions over push injections regarding VIPN development. Furthermore, due to low patient numbers, we were not able to study the effect of azole type, such as itraconazole, fluconazole, voriconazole, and posaconazole. This could very well be of importance as itraconazole is a stronger inhibitor of CYP3A4 than for instance fluconazole or voriconazole[51]. Finally, diagnostic indication for treatment with azole antifungals, such as prophylaxis or treatment of diagnosed mycoses, on the relation between administration method and VIPN should be considered as well in a future study.

Although VIPN is a serious toxicity, high therapeutic effectiveness of VCR is of utmost importance. Regarding administration method, it is not expected that one-hour infusions of VCR are associated with a worse therapeutic outcome than push injections. It might even be the contrary. In general, longer lasting infusions of chemotherapy may improve therapeutic efficacy of a drug with a short half-life and an action mechanism of the drug that is related to the cell cycle, both of which are true for VCR[26]. While it is conceivable, that one-hour infusions are too short to benefit from this cell-cycle dependence or prolonged half-life, it underlines the unlikeliness of lower therapeutic effectiveness of one-hour infusions of VCR. In our study we also did not find any differences regarding relapse or mortality between the two groups.

Our study had some limitations. First of all, we included a heterogeneous group of patients with multiple diseases, varying VCR dosing regimens and co-medications. These different co-medications could theoretically alter pharmacokinetics and thus VCR exposure and VIPN development. In order to further investigate the true effect of VCR administration duration on the development of VIPN, future studies including more uniform diagnostic study groups are needed. Furthermore, in treatment protocols for ALL and Hodgkin's lymphoma, the use of glucocorticoids is common practice[29,33]. Adverse effects of these agents could mimic symptoms of VIPN not attributed to VCR. However, we see a similar distribution of disease types in our two randomization groups, thereby ensuring similar distribution of co-medication. Sec-

ondly, the assessment of VIPN in children in general is difficult. Children of younger age are not able to verbally express their complaints. Therefore, for children aged <5 years VIPN assessment often relies on parent reports[40,52], which could introduce bias. The CTCAE lacks extensive physical examination, such as manual strength testing, vibration sense or assessment of sensibility, whereas the ped-mTNS cannot be used in younger children. As a consequence, both tools might not measure the same aspects of VIPN in the same population. Nevertheless, currently they represent the best available methods for VIPN assessment in children. Furthermore, assessors of VIPN in this study, although frequently unaware of randomization status of the patients, were not strictly blinded to the randomization status. This could have introduced bias in the VIPN results reported.

Finally, VIPN is a multifactorial phenomenon, also influenced by PK of VCR and single nucleotide polymorphisms[1]. It would be beneficial to study the impact of administration duration on VIPN while also considering these factors. Data on VCR PK and SNP's were also collected as part of this trial, but analyses of these data was beyond the scope of this paper. Data of this trial regarding administration duration related to PK were published separately[53]. Potentially, infusion of VCR in one-hour could lead to an increased risk of extravasation, which is dangerous in VCR treatment. However, in all our patients, VCR was administered using a central venous catheter, therefore, extravasation was not a potential risk factor.

CONCLUSIONS

In conclusion, our study showed that one-hour infusions of VCR are overall not associated with less VIPN compared to push administrations in pediatric cancer patients. However, when concurrent treatment with azole antifungals or similar medication affecting VCR-metabolism is necessary, VCR one-hour infusions seem to be beneficial over push injections, since these lead to less severe VIPN, although larger trials are needed to confirm this association.

SUPPLEMENTARY MATERIALS

Table S1: Baseline VIPN scores of patients of the intention-to-treat group for both randomization groups

Table S2: The effect of VCR administration method (push administration versus one-hour administration) on vincristine-induced peripheral neuropathy in the intention-to-treat analysis additionally corrected for age, sex, VCR dose per m^2, cancer diagnosis and ethnicity

Figure S1: Measurement schedule per treatment protocol, Supplementary Study protocol: Vincristine-Induced Neuropathy in children with CAncer (the VINCA study) version 5.3

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Population Pharmacokinetics of Vincristine Related to Infusion Duration and Peripheral Neuropathy in Pediatric Oncology Patients

M.E. van de Velde, J.C. Panetta, A.J. Wilhelm, M. H. van den Berg, I.M. van der Sluis, C. van den Bos, F.C.H. Abbink, M.M. van den Heuvel – Eibrink, H. Segers, C. Chantrain, J. van der Werff Ten Bosch, L. Willems, W.E. Evans and G.J.L. Kaspers

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ABSTRACT

Vincristine (VCR) is frequently used in pediatric oncology and can be administered intravenously through push injections or one-hour infusions. Effect of administration duration on population pharmacokinetics (PK) are unknown. We described PK differences related to administration duration and the relation between PK and VCR induced peripheral neuropathy (VIPN). PK was assessed in 1-5 occasions (1-8 samples in 24 hours per occasion). Samples were analyzed using high-performance liquid chromatography/tandem mass spectrometry. Population PK of VCR and relation with administration duration was determined using non-linear mixed effect. We estimated individual post-hoc parameters: area-under-the-concentration-time-curve (AUC) and maximum concentration (C_{max}) in the plasma and peripheral compartment. VIPN was assessed using Common Terminology Criteria for Adverse Events (CTCAE) and pediatric-modified Total Neuropathy Score (ped-mTNS). Overall, 70 PK assessments in 35 children were evaluated. Population estimated intercompartmental clearance (IC-Cl), volume of the peripheral compartment (V_2) and C_{max} were significantly higher in the push group. Furthermore, higher IC-Cl was significantly correlated with VIPN development. Administration of VCR by push led to increased IC-Cl, V_2 and C_{max}, but similar AUC compared to one-hour infusions. Administration of VCR by one-hour infusions led to similar or higher exposure of VCR without increasing VIPN.

INTRODUCTION

Vincristine (VCR) is a frequently used chemotherapeutic agent for the treatment of several types of pediatric malignancies[1]. It works by inhibiting mitosis[2,3] and VCR is primarily eliminated by metabolism in the liver via the cytochrome P450 (CYP) enzymes, particularly CYP3A4 and CYP3A5[4].

The development of a high-performance liquid chromatographic (HPLC) assay for VCR[5] has generated reliable data regarding the pharmacokinetics (PK) of VCR in children. Previous studies have shown a large inter- and intra-patient PK variability[6-10] and a higher clearance (Cl) rate in children compared to adults[6].

Previous studies investigating the relation between VCR PK and toxicity showed conflicting results, with some studies indicating that there is no association between PK and VCR induced peripheral neuropathy (VIPN)[6,8,11,12], the major dose-limiting toxicity of VCR, whereas others did report this association[1,13,14].

VIPN is caused by the disruption of microtubules within the cell, resulting in neuronal axon dysfunction. Also inflammatory processes seem to play a role in the pathophysiology of VIPN. Ultimately, it leads to a progressive motor, sensory and autonomic nerve damage due to dysfunction of Aβ, Aδ and C-fibers[14]. VIPN can lead to dose reductions or omitted doses[1,15,16] of VCR. In children, VCR doses higher than 2 mg/m^2 (with a maximum of 2mg) have shown to lead to intolerable VIPN[15]. However, Frost et al. suggested that the common practice of dose capping VCR at 2 or 2.5 mg should be carefully reconsidered, since they found no evidence to suggest age-dependent PK of VCR, indicating that by capping the VCR dose older children are less intensely treated than younger children[17]. The duration of VCR administration may also play a role in the development of VIPN. When continuous administrations of up to five consecutive days were used, doses up to 7.5 mg/m^2 without dose capping were well tolerated in children and adults[18,19]. However, it is evident that such long administration durations and high doses are costly and cumbersome and there is no clear indication of a better therapeutic efficacy[20]. Therefore, an alternative one-hour infusion is more feasible and may also offer a favorable toxicity profile compared to shorter administration durations. However, in clinical practice very short administration times, i.e. push injections lasting one to five minutes, are often the standard care for pediatric cancer patients. Administration time directly influences the maximum concentration (C_{max}), which was previously associated with VIPN[21]. However the effect of administration duration of VCR on its PK remains to be established, as well as how these PK are related to the development of VIPN in children.

In addition, a drug-drug interaction between VCR and concurrent treatment with many azole antifungals was previously reported[22-24]. These azole antifungals are frequently used during intensive chemotherapeutic treatment in children for the prevention or treatment of invasive fungal infections[25]. The interaction is based on the inhibiting effect of azole antifungals on CYP3A4 and P-glycoprotein[26]. However, possible associations between this drug-drug interaction and VCR PK are largely unknown[27].

To address these issues, the primary aims of the current study were: 1. to assess PK of VCR given to pediatric cancer patients as either push injections or one-hour infusions; 2. to investigate the association between PK and exposure parameters and VIPN; 3. to investigate the effect of concurrent azole antifungal treatment on VCR PK parameters.

MATERIALS AND METHODS

Patients

The current study is part of an RCT investigating the relation between administration method of VCR and the development of VIPN in children with cancer. Participants of this RCT received all planned VCR administrations of their treatment protocol either through intravenous push injections or through one-hour infusions. Children with the following diseases and treatment protocols were eligible for participation: acute lymphoblastic leukemia (ALL) (DCOG ALL-11 protocol[28], EORTC-58081-CLG guideline[29] or EsPhALL protocol[30]), Hodgkin lymphoma (EuroNet-PHL-C1 protocol[31] or C2 protocol[32]), rhabdomyosarcoma (EpSSG RMS 2005 protocol[33]), nephroblastoma (SIOP Wilms 2001 protocol[34]), medulloblastoma (ACNS0331[35] or ACNS0332[36] protocol), and low-grade glioma (SIOP LGG 2004 protocol[37]). Results of the RCT part of the trial are reported separately[38]. This trial was registered in the Dutch Trial Registry (www.trialregister.nl) with number NL4019.

Participants of the RCT could choose whether they also wanted to participate in the PK part of the study. Of those who agreed to participate, written informed consent was obtained from parents and/or children (in case the child was ≥12 years). Blood samples were collected between September 2014 and April 2018 in either one of the four Dutch or three Belgian participating centers. The study was approved by the Institutional Review Board of the Amsterdam UMC, location VUmc.

Pharmacokinetic sampling

VCR was administered to patients throughout treatment with a dose of either 1.5 mg/m^2 or 2 mg/m^2 (depending on standard care as defined by the concerning treatment protocol and treatment phase) with a maximum of 2 mg. A push injection was administered with the required dose diluted in an injection of 10 mL 0.9% NaCl or in an intravenous bag of 50 mL 0.9% NaCl. A push injection was defined as an administration time between 1 and 5 minutes. One hour-infusions were administered using an intravenous bag in which the required dose was diluted in 50 mL of NaCl 0.9%. Infusion rate of one-hour infusions depended on hospital: standard administration was in 60 minutes (bag of 50 mL VCR in 38 minutes plus flushing of the VCR filled line in 22 minutes), however in four patients from one hospital, total administration time was 96 minutes (bag of 50 mL in 60 minutes plus flushing of the VCR filled line in 36 minutes). Administration of push injections and one-hour infusions were done both through a central venous catheter either directly (push) or extended with a line (some of the push injections (depending on hospital) and all of one-hour infusions). Peripheral blood was sampled either using a separately placed peripheral cannula, or using

the central line after this was thoroughly rinsed according to a previously published protocol[39]. Peripheral blood (2 mL) was collected in Lithium Heparin tubes at 10, 20, 30, 40, 60, 75, 140 and, if the child was still in the hospital, 1440 minutes after start of VCR treatment. Depending on the length of the treatment protocol, samples were taken at 1-5 different occasions.

Plasma was separated by centrifuging the sample at 4000 rpm for 5 min and subsequently stored at −80 °C until analysis.

Assessment of VIPN

VIPN was assessed on the same day of PK sampling using two different instruments. Of the Common Toxicity Criteria of Adverse Events (version 4.03[40]) the items peripheral sensory neuropathy (score 0-5), peripheral motor neuropathy (score 0-5), constipation (score 0-5) and neuralgia (score 0-3) were used to calculate a VIPN sum score. A CTCAE item score of two or higher was defined as VIPN. Furthermore, the validated Dutch translated version of the pediatric modified total neuropathy score (ped-mTNS)[41,42] was used. This validated instrument, which includes both a questionnaire part (sensory, functional and autonomic symptoms) and a physical examination, has been developed to assess VIPN in children aged 5 years or older. As such, in the current study this instrument was not used in children below 5 years of age. A total ped-mTNS score of ≥ 5 was defined as VIPN[41].

Quantification of plasma VCR concentrations

Vincristine sulphate (C46H56N4O10*H2SO4) was purchased from Sequoia Research Products (Pangbourne, UK). High-performance liquid chromatography (HPLC) grade methanol and acetonitrile originated from Biosolve Ltd. (Amsterdam, The Netherlands). Ammonium acetate and ammonia 25% were from Merck (Darmstadt, Germany). Double distilled water was used throughout analysis.

Concentration of VCR in plasma samples were analyzed using HPLC/tandem mass spectrometry (HPLC/MS/MS) coupled with electrospray ionization (ESI), as reported previously[43]. Briefly, plasma samples of 30 µL were protein precipitated with acetonitrile/methanol (50:50, v/v) containing the internal standard vinorelbine and 7-AAD. After vortex-mixing and centrifugation, 10 µL of the supernatant was injected on the analytical column.. A Finnigan TSQ Quantum Ultra triple quadrupole mass spectrometer equipped with an ESI source (Thermo Fisher Scientific, Waltham, MA, USA) operating in the positive ion mode was used as a detector. Chromatographic separation of VCR and the internal standards was carried out using a LC-20AD Prominence binary solvent delivery system with a column oven, DGU-20A3 online degasser, and

a SiL-HTc controller (Shimadzu, Kyoto, Japan). The mobile phase was composed of a mixture 1 mM (70:30, v/v) ammonium acetate/acetonitrile adjusted to pH 10.5 using 25% ammonia and mobile phase B was methanol. Gradient elution was applied at a flow rate of 0.4 mL/min through an Xbridge C18 column (502.1mm i.d., particle size 5mm; Water, Milford, MA, USA) protected with a 0.5mm filter (Upchurch Scientific, Oak Harbor, WA, USA) and thermostatted at 40° C. Standards (0.25–100 ng/ml) were included in every batch of samples. HPLC run time was 6 min. The assay quantifies VCR concentrations in plasma from 0.25 to 100 ng/mL. Concentrations of VCR were expressed as ng/ml. Samples were appropriately diluted and re-analyzed when the concentrations were beyond the upper limit of quantification (ULOQ) of the assay.

Pharmacokinetic analysis

A linear two-compartment model with first-order elimination was used to describe the VCR concentration vs time data. Specifically, the model was fit to pharmacokinetic data from all individuals simultaneously using non-linear mixed-effects modeling (Monolix, version 5.1.0) with the stochastic approximation expectation-maximization (SAEM) method. The PK parameters estimated included apparent Cl (L/hr/m^2)) and volume of the central compartment (V_1 (L/m^2)) along with the inter-compartmental clearance (IC-Cl (L/hr/m^2) and volume of the peripheral compartment (V_2 (L/m^2)). The inter-individual and inter-occasion variability of the parameters was assumed to be log normally distributed. A proportional residual error model was used with assumed normal distribution of the residuals. In addition, the individual post-hoc PK estimates (Empirical Bayesian Estimates---EBE) were used to estimate the VCR plasma and peripheral AUC (0-3 hrs), C_{max}, and time above 1 ng/mL. A graphical depiction of our final model is shown in Figure 1.

Concurrent azole antifungal treatment

Data regarding concurrent azole antifungal treatment were collected from medical chart review. If the child used any type of azole antifungals in the week preceding or on the day of PK measurement, this measurement was considered as performed during concurrent azole antifungal treatment.

Statistical analysis

Covariate analysis were handled either directly in the population pharmacokinetic modeling for the primary model parameters or using the two-stage approach for the secondary exposure parameters. In the population PK analysis, covariates were considered significant in a univariate analysis if their addition to the model reduced the objective function value (OFV) by at least 3.84 units ($p < 0.05$, based on the chi-square test for the difference in the −2 log-likelihood between two hierarchical models that

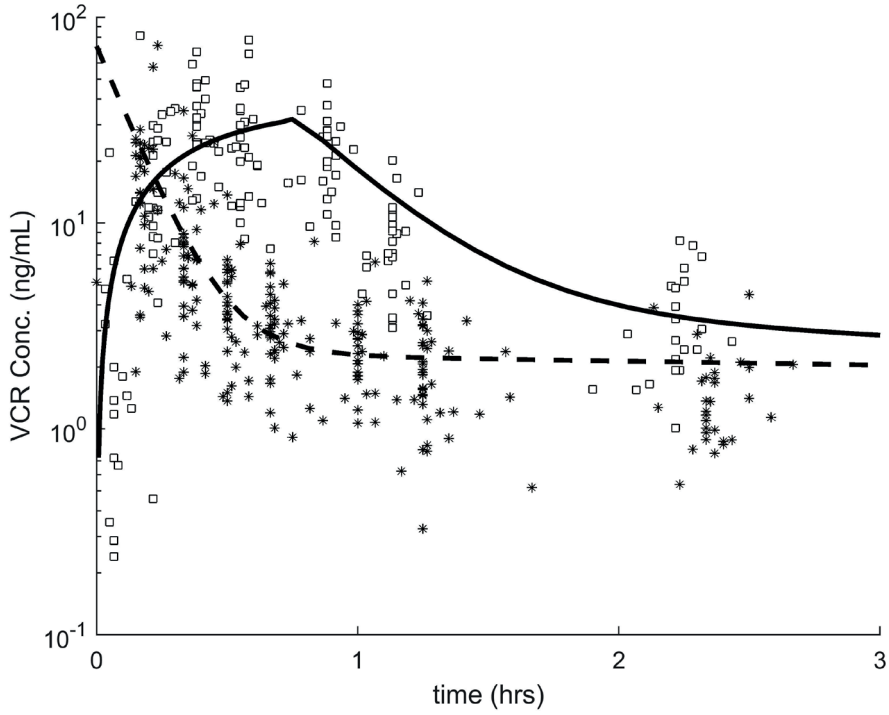

Figure 1. Two-Compartment Pharmacokinetic Model.
Cl: apparent clearance (L/hr/m^2)); V_1: volume of the central compartment (L/m^2); IC-Cl: inter-compartmental clearance (L/hr/m^2); and V_2: volume of the peripheral compartment (L/m^2). push data; dashed black curve: model fit to the push data. Black squares: 1 h infusion data; solid black curve: model fit to the 1 h infusion data; black stars: push data; dashed black curve: model fit to the push data.

differ by 1 degree of freedom) and the covariate term was significantly different from zero ($p < 0.05$ by a t-test). The two-stage approach, used to evaluate the covariate effects on the exposure measures, was performed as follows: 1.) estimate population PK, generate the individual post-hoc PK parameters (EBE), and calculate each occasion's measures of exposure using its EBE; 2.) use linear mixed-effects models to evaluate the effects of the covariates on the individual exposure estimates. The studied covariates using this approach were age, sex, VCR dose per m^2, disease, concurrent azole antifungal treatment and self-declared ancestry.

Relation between PK parameters and VIPN were analyzed using mixed effect models for continuous outcomes. Dichotomous outcomes were analyzed using logistic generalized estimating equations (GEE). By using multivariate analysis, results were corrected for age, sex, VCR dose per m^2, disease, concurrent azole antifungal treatment and self-declared ancestry. Descriptive data of normally distributed variables

were reported as mean ± SD and skewed variables as median and interquartile range. A two-tailed p value of <0.05 was considered statistically significant. Statistical analysis regarding the relation between PK and VIPN were performed using SPSS 26.0 (Chicago, IL, USA).

RESULTS

Study population

In total, 35 out of 90 patients enrolled in a randomized clinical trial (RCT) also participated in the PK studies. Table 1 shows the characteristics of these 35 patients, which represented the characteristics of the entire cohort of 90 patients. In total, 20 patients (57%) received their VCR through push injections, while one-hour infusions were given in 15 patients (43%). All patients in the study received the standardized dose of 1.5 mg/m^2 or 2 mg/m^2 with a maximum of 2 mg. In total, PK measurements were performed on 70 different occasions (1-5 per patient), which resulted in a total of 425 samples. Doses were capped at 2 mg in 20 patients (37 occasions). At two of the 70 occasions (3%), two patients received a 15 minute VCR infusion instead of the administration method according to their randomization. These were analyzed as push injections.

Table 1. Patient characteristics of the included patients

	Pharmacokinetic part of trial			RCT part of trial
	Total (n=35)	**Push group (n=20)**	**One-hour group (n=15)**	**Total group (n=90)**
Age in years, mean (SD)	10.06 (5.6)	10.30 (6.17)	9.73 (4.85)	9.17 (5.15)
Ancestry, n (%)				
Caucasian	30 (86)	17 (85)	13 (87)	73 (81)
Non-Caucasian	5 (14)	3 (15)	2 (13)	17 (19)
Sex, n (%)				
Male	16 (45)	9 (45)	7 (47)	50 (56)
Female	19 (54)	11 (55)	8 (53)	40 (44)
Diagnosis, n (%)				
ALL	26 (74)	12 (60)	14 (93)	58 (64)
Hodgkin	6 (17)	5 (25)	1 (7)	18 (20)
Medulloblastoma	1 (3)	1 (5)	0 (0)	2 (2)
LGG	1 (3)	1 (5)	0 (0)	2 (2)
Wilms	1 (3)	1 (5)	0 (0)	8 (9)
RMS	0 (0)	0 (0)	0 (0)	2 (2)

RCT: randomized clinical trial; SD: standard deviation, ALL: acute lymphoblastic leukemia, LGG: low-grade glioma.

Population Pharmacokinetics of VCR

The population PK for VCR are summarized in Table 2 and Figure 2. First we considered the effects of VCR administration method (push vs 1-hr infusion). We observed that there were significant differences in the PK parameters that affect the terminal phase. Specifically, IC-Cl and V_2 were each 3.1 times higher in the push vs 1-hr infusion group (p-5.1e-9; Table 2). While there was a trend to lower VCR Cl in the push group, this difference did not reach significance (p=0.058). The Individual Weighted residuals of our final model can be found in Figure 3.

Table 2. Population pharmacokinetics of vincristine

	Base Model		Administration Type	
	Population Estimate	**RSE (%)**	**Population Estimate**	**RSE (%)**
Cl (L/hr/m²)	25.3	10.1	30.5	10.0
V_1 (L/m²)	22.1	14.4	20.8	12.5
IC-Cl (L/hr/m²)	77.9	12.8	34.2	9.5
beta on IC-CL (push)			1.13	4.5
V_2 (L/m²)	358.5	11.3	127.7	19.0
beta on V_2 (push)			1.13	19.1
Inter-Individual Variability	CV%		CV%	
Cl (L/hr/m²)	0.51	17.4	0.52	16.9
V_1 (L/m²)	0.62	20.7	0.55	23.9
IC-Cl (L/hr/m²)	0.67	16.7	0.48	16.4
V_2 (L/m²)	0.48	17.4	0.41	17.0
Residual (CV%)	0.46	4.4	0.45	4.3
-2 Log-likelihood	2212.5		2174.3	

Base Model: model not considering any covariates, Administration Type: model accounting for the effects of the method of administration (1-hr infusion vs push) on the pharmacokinetic parameters, RSE: relative standard error, Cl: clearance, V_1: volume of distribution of the central compartment, IC-Cl: inter-compartmental clearance, V_2: volume of distribution of the peripheral compartment, CV%: coefficient of variation, Covariate model: $PK=PK_{pop}$*exp(beta*push) where push=0 if 1-hr infusion and 1 if push.

Table 3. Post-hoc pharmacokinetics of vincristine per administration method additionally corrected for age, sex, VCR dose per m², disease, concurrent azole antifungal treatment and self-declared ancestry.

	Total group		One-hour infusions		Push injections		Comparison of push injections versus push one-hour infusions		
	Median	**IQR**	**Median**	**IQR**	Median	IQR	Beta (95% CI)*	95% CI*	P-value
Plasma AUC (ng*hr)/mL	39.78	31.91 – 49.47	44.04	35.52 – 57.17	38.60	30.54 – 43.66	-2.13	-7.97 to 3.71	0.46
Peripheral AUC (ng*hr)/mL	36.71	27.48 – 45.78	42.50	33.78 – 55.75	35.36	26.22 – 40.97	-4.18	-10.26 to 1.90	0.17
Plasma C_{max} ng/mL	57.28	31.91 – 74.47	30.05	21.86 – 39.07	72.44	58.64 – 86.12	43.01	31.55 to 54.47	<0.001
Peripheral C_{max} ng/mL	2.87	2.24 – 4.37	4.81	3.15 – 6.94	2.57	1.77 – 2.92	-1.79	-2.63 to -0.94	<0.001

IQR: interquartile range, 95% CI: 95% confidence interval, AUC: area under the concentration time curve, C_{max}: maximum concentration

*one-hour = reference group, beta represents the difference of the corresponding parameter between the two groups.

We also considered the effects of azole antifungal treatment on the VCR PK but did not find any significant relationships.

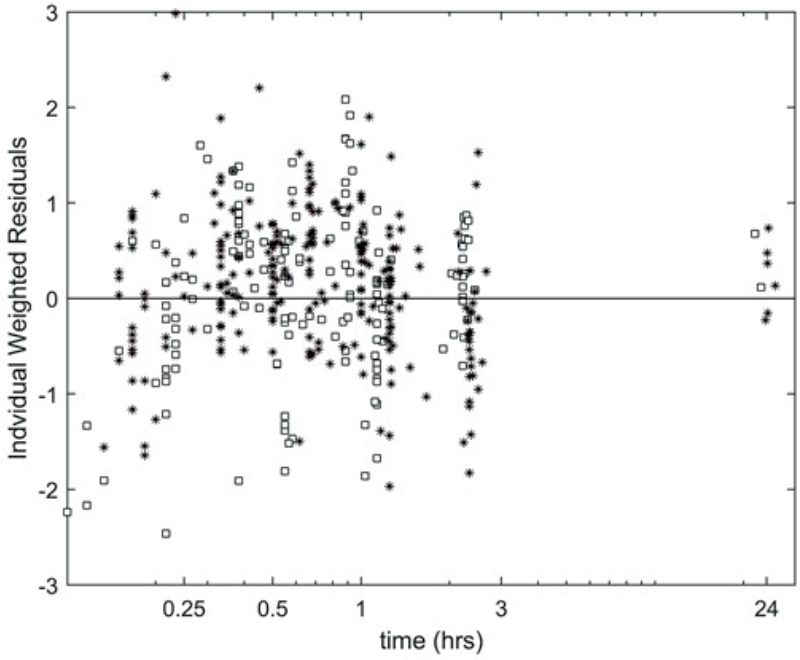

Figure 2. Individual weighted residuals for the final model.
Black Squares: 1 h infusion data; black stars: push data.

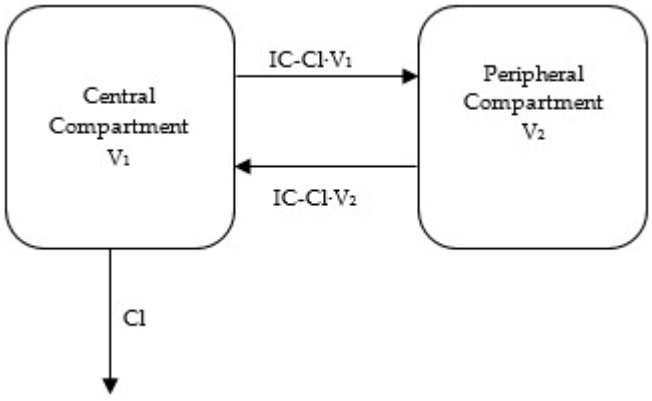

Figure 3. Two-compartment pharmacokinetic model.
Cl: apparent clearance (L/h/m2)); V1: volume of the central compartment (L/m2);

Table 4. Association between pharmacokinetic parameters and vincristine induced peripheral neuropathy additionally corrected for age, sex, VCR dose per m^2, disease, concurrent azole-antifungal treatment and self-declared ancestry.

	Total CTCAE score		Total ped-mTNS score		VIPN CTCAE score no/yes*		VIPN ped-mTNS score no/yes*	
	Beta (95%CI)	P value	Beta (95%CI)	P value	OR (95% CI)	P-value	OR (95% CI)	P-value
Cl ($L/hr/m^2$)	0.00 (-0.04 to 0.05)	0.90	0.02 (-0.09 to 0.14)	0.70	1.01 (0.96 to 1.06)	0.74	1.05 (0.99 to 1.12)	0.11
V_1 (L/m^2)	-0.00 (-0.06 to 0.09)	0.96	-0.03 (-0.19 to 0.13)	0.72	1.00 (0.89 to 1.11)	0.93	0.98 (0.91 to 1.07)	0.98
IC-Cl ($L/hr/m^2$)	0.01 (0.00 to 0.02)	0.04	0.04 (0.01 to 0.07)	0.004	1.02 (1.00 to 1.03)	0.02	1.05 (1.02 to 1.09)	0.001
V_2 (L/m^2)	-0.00 (-0.00 to 0.00)	0.88	0.01 (-0.00 to 0.02)	0.20	1.00 (0.99 to 1.00)	0.29	1.00 (1.00 to 1.01)	0.81
Plasma AUC (ng*hr/mL)	-0.01 (-0.06 to 0.03)	0.54	-0.06 (-0.16 to 0.05)	0.27	0.97 (0.93 to 1.02)	0.21	0.95 (0.89 to 1.02)	0.18
Peripheral AUC (ng*hr/mL)	-0.01 (-0.05 to 0.04)	0.76	-0.05 (-0.16 to 0.06)	0.34	0.98 (0.93 to 1.03)	0.40	0.97 (0.91 to 1.04)	0.41
Plasma C_{max} (ng/mL)	-0.01 (-0.03 to 0.02)	0.55	0.00 (-0.08 to 0.07)	0.94	0.96 (0.92 to 1.00)	0.05	0.99 (0.96 to 1.02)	0.53
Peripheral C_{max} (ng/mL)	-0.00 (-0.36 to 0.35)	0.99	-0.31 (-1.27 to 0.65)	0.52	0.97 (0.64 to 1.47)	0.89	0.96 (0.61 to 1.49	0.85

CTCAE: common toxicity criteria of adverse event, ped-mTNS: pediatric modified Total Neuropathy Score, VIPN: vincristine induced peripheral neuropathy, Cl: clearance, 95% CI: 95% confidence interval, V_1: volume of distribution of the central compartment, IC-Cl: intercompartmental clearance, V_2: volume of distribution of the peripheral compartment, AUC: area under the concentration time curve, C_{max}: maximum concentration
*no VIPN as reference.

In addition, we evaluated differences in VCR exposure due to administration method. We observed higher, although not statistically significant, AUC in the 1-hr infusion group (median plasma AUC one-hour group: 44.04 (ng*hr)/mL, median plasma AUC push group: 38.60 (ng*hr)/mL, p=0.22, median peripheral AUC one hour group: 42.50 (ng*hr)/mL, median peripheral AUC push group: 35.36 (ng*hr)/mL, Table 3). In addition, we observed significantly higher plasma Cmax but significantly lower peripheral

C_{max} in the push group compared to the one-hour group (median plasma C_{max} > 2 times higher in the push group; p = <0.001, median peripheral C_{max} 2 times lower in the push group; p<0.001, Table 3). Finally, median time with a plasma VCR concentration above 1 ng/mL differed between the two administration groups: one-hour group median time = 0.92 h, push group median time = 0.24 h; p<0.001 as well as the median time with an estimated peripheral VCR concentration above 1 ng/mL (one-hour median time: = 14.65 h, push median time = 16.83 h; p=0.001).

Association between PK parameters and VIPN

Both the total CTCAE and ped-mTNS scores and the dichotomized CTCAE and ped-mTNS scores were significantly associated with IC-Cl, with higher IC-Cl associated with VIPN (see Table 4). All other primary parameters (Cl, V_1 and V_2) as well as post-hoc parameters (plasma AUC and C_{max} and peripheral AUC and C_{max}) were not associated with any of the VIPN outcomes.

DISCUSSION

This study evaluated the effects of administration duration (push injections versus one-hour infusions) on the pharmacokinetics of VCR in children with cancer along with the effects of VCR PK on VIPN. We observed that the intercompartmental clearance (IC-Cl), volume of the peripheral compartment (V_2), and plasma and peripheral C_{max} were significantly higher in the push group compared to individuals in the one-hour group. Finally, we showed that higher IC-Cl was significantly correlated with VIPN development.

When VCR PK was previously studied, VCR was generally administered as a push injection. In these studies mean Cl of VCR push injections varied between 13.44 and 28.9 L/hr/m^2 [6-8,17,20] whereas Cl of our push cohort was: mean [range]: 26.86 [13.45 – 48.40] L/hr/m^2. However, the mean Cl of the one-hour cohort was mean [range]: 32.61 [19.76 – 72.67] L/hr/m^2, which is higher than previously published data of Cl in push injected VCR and corresponds well with our nearly statistically significant finding of higher Cl in the one-hour group compared to the push injection group. In addition, in our push cohort median plasma AUC was slightly lower than previously published median plasma AUC (46.17 - 111.89 (ng*hr)/mL[7,17,20]), however our range of measured AUC's [17.22 – 63.05 (ng*hr)/mL] is in-line with these other median values of AUC. One previous VCR PK study by Guilhaumou et al.[8] (VCR administered as push injection) reported values of IC-Cl (51.9 L/hr/m^2), V_1 (15.9 L/m^2) and V_2 (145 L/m^2), which differed from the results in our push cohort: median [range]: IC-CL: 129.73 [30.76 – 301.56] L/hr/m^2, V_1: 19.57 [9.03 – 34.97] L/m^2 and V^2: 445.57 [115.08 – 803.58] L/m^2.

To the best of our knowledge, this is the first study that aimed to establish the association between administration duration of VCR and PK parameters. However, there are multiple studies that have investigated the effect of prolongation of administration duration on PK in other drugs. One study reported the effects of 1-minute versus 1-hour and 3-hour administrations of midazolam. Just like VCR, midazolam is metabolized in the liver through the CYP3A4 family of enzymes[44]. Results of this study showed that Cl was significantly higher when administered in 1-minute compared to an administration time of 1-hour or 3-hours ($p<0.001$)[45]. In our study, we also found a nearly statistically significant difference in Cl related to administration duration, and a significant difference of IC-Cl. The effect of administration duration on PK measures was also studied in children using ifosfamide, another CYP3A mediated agent[46]. In this study, continuous infusions (72 hours) were compared with one-hour infusions. It showed a higher Cl of ifosfamide in the continuous infusion group compared to the

one-hour group, whereas we observed no such difference. However, AUC was similar between the two administration groups[47], which was comparable to our results.

In our study, we observed that higher IC-Cl was significantly associated with the development of VIPN. In previous studies, as mentioned earlier, results regarding the association between PK parameters of VCR and VIPN were conflicting[6,11,13,14,48]. In one study a positive association between VCR AUC and the concentration of metabolite M1 and VIPN was identified, unfortunately it is unknown what the VCR administration duration was in this trial[13]. However, in the study by Crom et al., in which VCR was administered as a push injection, no significant association between VCR AUC and VIPN was found[6]. In addition, Moore et al. also found no significant associations between PK parameters and VIPN. In this study VCR was administered in 5-10 minutes[11]. Guilhaumou et al. studied IC-Cl, terminal half-life, Cl and AUC of VCR push injections in relation to VIPN and also found none of these parameters to be significantly associated[48]. Finally, Plasschaert et al. studied the relation between Cl, AUC and distribution and elimination half-life of VCR push injections and constipation, as a marker for VIPN, and did not find any significant associations[12]. All these studies that did not find a significant association between PK and VIPN are in contrast to our findings. This could be due to the fact that we used two different instruments to prospectively assess VIPN. In particular, ped-mTNS was not available when the other studies were performed. It is considered a more sensitive tool in assessing VIPN in children than the commonly used CTCAE criteria[49]. Therefore, we may have detected more subtle symptoms of VIPN compared to these previous studies and thus were able to find an association between IC-Cl and VIPN. Furthermore, in four out of six of these studies the assessment of VIPN was done retrospectively through medical chart examination[6,11-13], whereas we assessed VIPN prospectively.

It has been previously reported that azole antifungal treatment concurrent with VCR dosing influenced the development of VIPN[22-24]. However, our study does not show any relationship between VCR PK and azole treatment. This is possibly due to our limited sample size (only 7/70 (n=6 patients) measurements were with concurrent azole antifungal treatment). Furthermore, we used any type of azole antifungal to study the relationship with concurrent VCR use, even though there are differences in the CYP450 inhibiting effect between different azole antifungals and therefore effects of concurrent VCR-azole antifungal use should ideally be studied for each different type of azole antifungal. Previously, Hasselt et al.[50] reported that a minimum sample size of 38 patients with at least 150 samples was required to detect a Cl inhibition of concurrent azole and VCR treatment with 80% power. This suggests our data are not powered to detect significant changes in VCR PK due to concurrent azole treatment.

We acknowledge some limitations to this study. First of all, our patient participation was limited compared to our entire cohort, which was probably due to the fact that sampling could not be done by capillary blood drawings or in many occasions through a central line, since this line was used for the administration of VCR in the one-hour group during sampling. Therefore, an extra peripheral cannula had to be inserted for trial participation. This was invasive and led to refusal of the PK part of our study for some participants and withdrawing of informed consent during the study, thereby lowering our participating patient numbers. Despite these dropouts our sample size remained reasonably large. Secondly, during a few PK sampling occasions different VCR administration durations were applied in one of the participating hospitals, but we allowed different administration durations within the final covariate model.

CONCLUSIONS

In conclusion, the current study showed that intercompartmental clearance (IC-Cl), volume of the peripheral compartment (V_2), and plasma and peripheral C_{max} were significantly associated with administration method of VCR in children with cancer. By prolonging VCR infusion from push injections to one-hour infusions, VCR treatment exposure remained similar, while lowering IC-Cl. A higher IC-Cl was significantly associated with VIPN. Therefore, prolonging VCR infusions is a good strategy to maintain similar or even higher exposure of VCR, without increasing the rate of VIPN.

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Genetic Polymorphisms Associated with Vincristine Pharmacokinetics and Vincristine-Induced Peripheral Neuropathy in Pediatric Oncology Patients

Mirjam E. van de Velde†, Aniek Uittenboogaard†, Wenjian Yang, Erik Bonten, Cheng Cheng, Deqing Pei, Marleen H. van den Berg, Inge M. van der Sluis, Cor van den Bos, Floor C.H. Abbink, Marry M. van den Heuvel–Eibrink, Heidi Segers, Christophe Chantrain, Jutte van der Werff ten Bosch, Leen Willems, William E. Evans† and Gertjan J.L. Kaspers†
†These authors contributed equally to this work

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ABSTRACT

Vincristine (VCR) is an important component of curative chemotherapy for many childhood cancers. Its main side effect is VCR-induced peripheral neuropathy (VIPN), a dose limiting toxicity. Some children are more susceptible to VIPN, which is at least partially dependent on genetic factors and pharmacokinetics (PK). In this study, we identify and replicate genetic variants associated with VCR PK and VIPN. Patient samples from a randomized clinical trial studying the effect of administration duration of VCR on VIPN in 90 patients were used. PK sampling was conducted on between one and five occasions at multiple time points. A linear two-compartment model with first-order elimination was used, and targeted next-generation DNA sequencing was performed. Genotype–trait associations were analyzed using mixed-effect models or logistic regression analysis for repeated measures, or Poisson regression analysis in which the highest VIPN score per patient was included. Nine single-nucleotide polymorphisms (SNPs) in seven genes (NDRG1, GARS, FIG4, FGD4, SEPTIN9, CEP72 and ETAA1) were associated with VIPN. Furthermore, three SNPs in three genes (MTNR1B, RAB7A and SNU13) were associated with PK of VCR. In conclusion, PK of VCR and VIPN are influenced by SNPs; upfront identification of those that lead to an altered susceptibility to VIPN or VCR exposure could help individualize VCR treatment.

INTRODUCTION

Vincristine (VCR) is one of the oldest and most widely used chemotherapeutic agents in pediatric oncology. It works by inhibiting mitosis by restriction of microtubule formation in the mitotic spindle[1,2]. It is metabolized in the liver by the cytochrome P450 (CYP) family of enzymes into the active metabolite M1, mainly by CYP3A4 and CYP3A5. The main dose limiting type of toxicity is VCR-induced peripheral neuropathy (VIPN). It is a mixed motor and sensory neuropathy, affecting the distal part of the longer nerves (i.e., feet and hands) and progressing more proximally when the condition worsens. It causes symptoms of paresthesia, pain, numbness or muscle weakness, among others. It also affects the autonomic nervous system where it can lead to, for example, constipation. VIPN is associated with a lower quality of life, both in patients undergoing treatment and in survivors of childhood cancer[3,4].

There are several factors that influence the development of VIPN in children receiving VCR. In terms of administration related factors, it was shown that a single dose exceeding 1.5–2 mg/m^2 with a maximum of 2 mg resulted in intolerable toxicity in general[5]. Furthermore, the development of VIPN is influenced by administration duration of VCR[6,7] and the individual pharmacokinetics (PK) of VCR[8,9]. In terms of patient related factors, older age appears to be associated with a higher risk of VIPN, although findings have been conflicting[2]. The relation between sex and VIPN remains unclear[2]. Moreover, it was shown that ancestry is associated with VIPN development, with studies showing that Caucasian children are more often affected than African(-American) children[10,11]. It was thus hypothesized that genomic factors are associated with VIPN[11]. Initially, this research focused on genomic differences between the CYP3A4 and CYP3A5 enzymes, since there was an ancestry dependent difference in the distribution of protein expression (i.e., African–American children express more CYP3A5 and Caucasian children express more CYP3A4). CYP3A5 was associated with faster metabolization and lower exposure to VCR[10,12-16], which could possibly explain the lower rate of VIPN in African–American children. However, these results were conflicting and could not fully explain the individual differences between VIPN[11]. In 2015, a genome-wide association study (GWAS) was published that identified a single-nucleotide polymorphism (SNP) in the gene coding for the Centrosomal Protein 72 (CEP72) that was associated with VIPN development[5]. Other studies have replicated this association since then[17,18]. To summarize all pharmacogenomic parameters associated with VIPN, we recently performed a systematic review and meta-analysis in which we found that SNPs in transporter-, metabolism-, cytoskeleton-, and hereditary neuropathy-associated genes were associated with VIPN[11].

Although the relation between genomic factors and VIPN has frequently been studied, all associated factors cannot fully explain the individual differences in the development of VIPN. The exposure of VCR after an administration is also determined by pharmacokinetics (PK), which is in turn affected by genetic factors. However, the direct effect of genetic variants on PK of VCR has not yet been studied. Understanding the association between genetic factors and PK is important, as it could lead to an individualized dosing regimen of VCR[9,11,19]. For example, children with a genetic variant associated with a higher exposure to VCR could benefit from dose reductions, whereas children who have a lower exposure might not benefit from the generally applied dose capping. By taking both PK and genetic associations into account, the individual dose of VCR could be optimized, while chances of developing VIPN are minimized[19]. Therefore, the goal of our study was to investigate the association between genetic factors and PK of VCR and to replicate or identify genetic factors associated with VIPN in pediatric oncology patients.

MATERIALS AND METHODS

Patients

Data of patients were collected as part of a randomized controlled clinical trial (RCT) which aimed to study the association between administration duration of VCR and VIPN in pediatric oncology patients. The design and results of this randomization are described elsewhere[20]. Briefly, participants of this RCT received all planned VCR administrations of their treatment protocol either through intravenous push injections (in 1–5 min) or through one-hour infusions. Children with the following diseases and treatment protocols were eligible for participation: acute lymphoblastic leukemia (ALL) (DCOG ALL-11 protocol 11 protocol[21], EORTC-58081-CLG guideline[22] or EsPhALL protocol[23]), Hodgkin lymphoma (EuroNet-PHL-C1 protocol[24] or C2 protocol[25]), rhabdomyosarcoma (EpSSG RMS 2005 protocol[26]), nephroblastoma (SIOP Wilms 2001 protocol[27]), medulloblastoma (ACNS0331[28] or ACNS0332[29] protocol), and low-grade glioma (SIOP LGG 2004 protocol[30]). Blood samples for PK analysis were collected between September 2014 and April 2018 in either one of four Dutch or three Belgian participating centers. The study was approved by the Institutional Review Board of the VUmc. Of all participants, written informed consent was obtained from parents and/or children (in case the child was ≥12 years). Participants of the RCT could choose and declare on the informed consent form whether they also wanted to participate in the PK part of the study.

Genomic Analysis

During treatment germline DNA of each participant was collected as specified in the study protocol. Whole blood samples were collected in PAXgene DNA collection tubes (Qiagen, Mississauga, ON, Canada). DNA was isolated and purified using the PAXgene Blood DNA Kit (Qiagen, Mississauga, ON, Canada) according to the manufacturer's instruction. DNA was analyzed using a targeted approach studying 48 candidate genes. These genes were selected based on a previously studied relation with VIPN, a relation with Charcot-Marie Tooth (CMT) (an inherited form of peripheral neuropathy which can worsen after VCR administration), possible relation with VCR PK, or possible association with VIPN based on the function of the expressed gene. These 48 genes are displayed in Supplementary materials 1. For each candidate gene, we included all exons and putative regulatory regions of 10,000 base pairs flanking the gene for targeted sequencing (Supplementary materials 1). Therefore, in this discovery and replication study, both SNPs that have previously been described in relation to VIPN and new SNPs were studied. The putative regulatory regions were selected based on ENCODE data version 3. Specifically, we extracted enhancer, transcriptional factor binding, and DNAse I hypersensitive regions for each cell line in ENCODE. Those

regions presenting all three features in multiple cell lines were selected. In addition, we also included regions 500 bp surrounding cis-acting expression quantitative trait loci (eQTLs) from the GTEx Portal (version 6, *p*-value < 10^{-7}). Roche NimbleGene SeqCap EZ probes were designed to capture these regions (Roche, Roche NimbleGen, Madison, WI, USA). Illumina HiSeq 2000 was used for paired-end sequencing with 100 bp read length. Sequencing reads in FASTQ format were mapped and aligned using the Burrows-Wheeler Aligner, and genetic variations were jointly called following GATK best practice version 3.8. Only those genotypes passing GATK quality control and exhibiting call rates greater than 95% were included for further association analysis. Linkage disequilibrium (LD) pruning was performed using the 'plink' package ($r^2 \geq 0.30$)[31,32]. The estimated impact of missense variants on protein function was estimated in silico using the scaled Combined Annotation Dependent Depletion (CADD) score[33] and the Rare Exome Variant Ensemble Learner (REVEL) score[34]. For intronic SNPs, the predicted impact on splicing was estimated in silico using the deep-learning-based tool SpliceAI[35].

Pharmacokinetic Analysis

Methods and results of the PK analysis were previously published[36]. Briefly, blood samples were collected at 10, 20, 30, 40, 60, 75, 140 and, if the child was still in the hospital, 1440 min after start of VCR treatment. Depending on the length of the treatment protocol, samples were taken at 1-5 different occasions during the treatment period. Samples were analyzed using high-performance liquid chromatography (HPLC)/tandem mass spectrometry (HPLC/MS/MS). The assay quantifies VCR concentrations in plasma from 0.25 to 100 ng/mL. A linear two-compartment model with first-order elimination was used to describe the VCR concentration vs. time data. The model was fit to PK data from all individuals simultaneously using non-linear mixed-effect modeling (Monolix, version 5.1.0 with the stochastic approximation expectation-maximization (SAEM) method. For this study, the included individual post-hoc PK estimates (Empirical Bayesian Estimates (EBE)) were used to estimate the plasma VCR area under the concentration time curve (AUC) and maximum VCR concentration (C_{max}).

Assessment of VIPN

VIPN was assessed prospectively at between one and five occasions during treatment using two different instruments. Of the Common Toxicity Criteria of Adverse Events (CTCAE) (version 4.03[37]) the items peripheral sensory neuropathy (grade 0–5), peripheral motor neuropathy (grade 0–5), constipation (grade 0–5) and neuralgia (grade 0–3) were used to calculate a VIPN sum score. A CTCAE item score of two or higher was defined as VIPN. Furthermore, the Dutch translated version of the pediatric-modified

Total Neuropathy Score (ped-mTNS)[38] was used. This validated instrument, which includes both a questionnaire part (sensory, functional and autonomic symptoms) and a physical examination, has been developed to assess VIPN in children aged 5 years or older. As such, in the current study this instrument was not used in children below 5 years of age. A total ped-mTNS score of ≥5 was defined as VIPN[38].

Statistical Analysis

VCR plasma PK parameters C_{max} and AUC were generated longitudinally at multiple occasions. Their associations with SNP genotypes were analyzed by mixed-effect linear models for repeated (longitudinal) measurements with genotype as the main effect of interest and a random effect with compound-symmetry intra-subject correlation structure. For a clear visualization, gross associations between the PK parameters and genotypes were displayed in box plots by pooling all measurements together ignoring time (Figure 1). Furthermore, VIPN was measured in two ways: according to the CTCAE, defined as the sum of the CTCAE grades of all VIPN-related toxicities, and the ped-mTNS. Mixed-effect Poisson regression models were fitted for the highest total CTCAE and total ped-mTNS scores per patient across the time points with SNP genotypes as the main effect of interest, and the baseline total CTCAE or ped-mTNS score as a covariate. For clear visualization, the total CTCAE and ped-mTNS scores across the different time points were displayed by boxplots according to genotypes (Figures 2 and S1). Second, the dichotomized VIPN scores (VIPN yes/no, defined CTCAE ≥ grade 2 on VIPN-related toxicities) were modeled by mixed-effect logistic regression, including the baseline CTCAE or ped-mTNS score as a covariate, and with a random effect

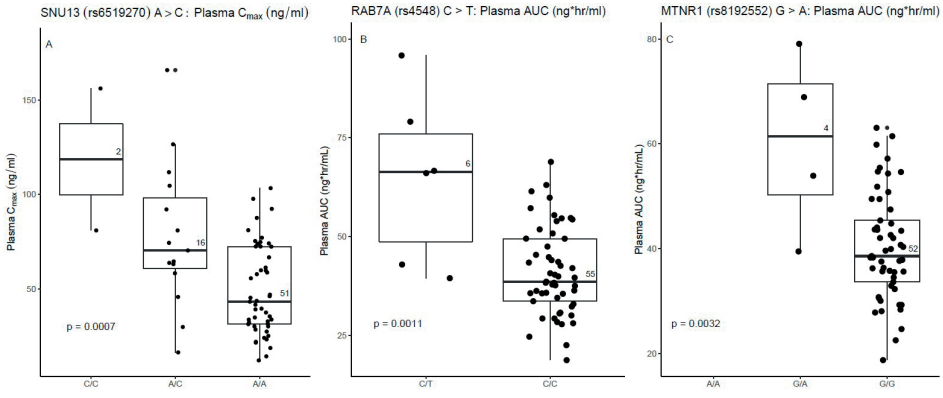

Figure 1. A: Association between a SNP in SNU13 and vincristine (VCR) pharmacokinetics (PK). B: Association between a SNP in RAB7A and VCR PK. C: Association between a SNP in MTNR1 and VCR PK.
PK samples were collected in 35 patients on maximum 70 occasions; every occasion per patient is shown. The number in the boxplot indicates the number of observations per genotype. The p-value was derived from mixed-effect linear regression for repeated measures, where the genotype was considered to be a categorical variable. SNU13: Small Nuclear Ribonucleoprotein 13, RAB7A: RAS-related protein 7A, MTNR1B: Melatonin Receptor 1B.

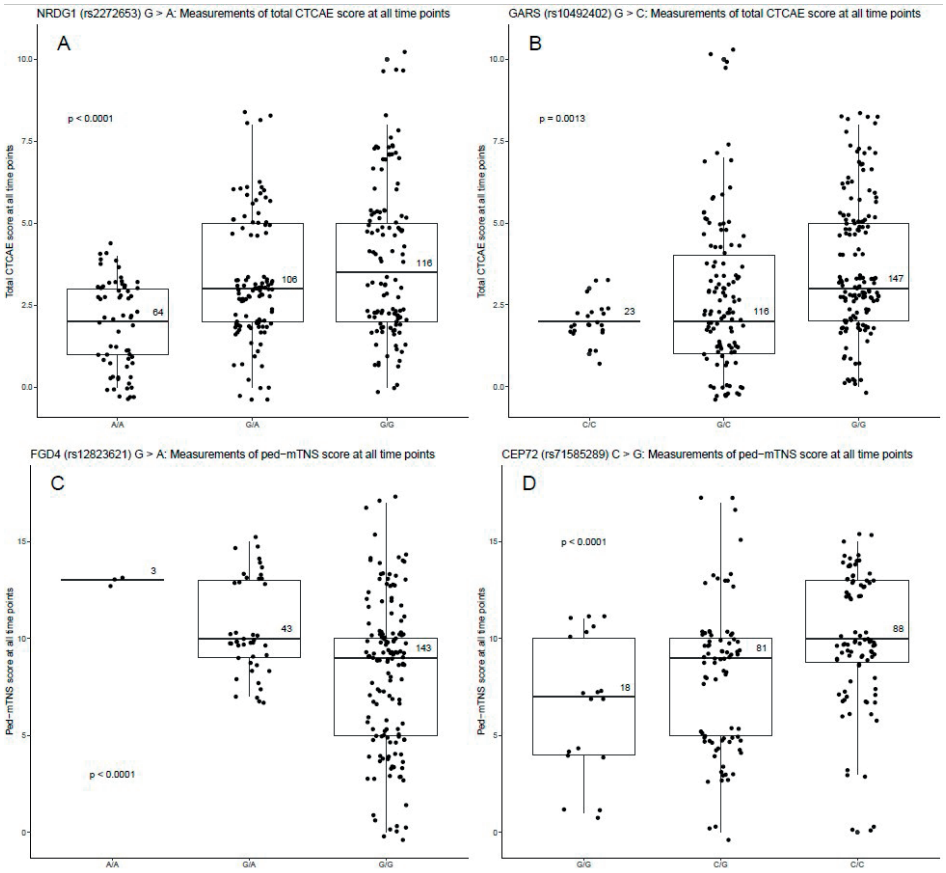

Figure 2. A: Association between a SNP in NRDG1 and total Common Toxicity Criteria of Adverse Events (CTCAE) score. B: Association between a SNP in GARS and total CTCAE score. C: Association between a SNP in FGD4 and pediatric-modified Total Neuropathy Score (ped-mTNS). D: Association between a SNP in CEP72 and ped-mTNS score. VIPN measurements were performed 1–5 times in 85 patients. Every VIPN measurement per patient across the time points is shown. The number in the boxplot indicates the number of observations per genotype. The p-value was derived from Poisson regression analysis for repeated measures, where the genotype was considered to be a categorical variable. NRDG1: N-Myc Downstream Regulated 1, GARS: Glycyl tRNA Synthetase, FGD4: FYVE, RhoGEF and PH Domain Containing 4, CEP72: Centrosomal Protein 72.

to account for intra-subject correlation. Again, for clear visualization, the number of VIPN assessments (yes/no) per patient were shown in boxplots according to genotype (Figure 3). To correct for multiple hypothesis testing, a false discovery rate (FDR) correction was applied to determine a significance threshold ($p < 0.004$, FDR = 23%)[39]. A multivariable analysis was performed, in which the additional variables disease, cumulative VCR dosage, and ancestry were included.

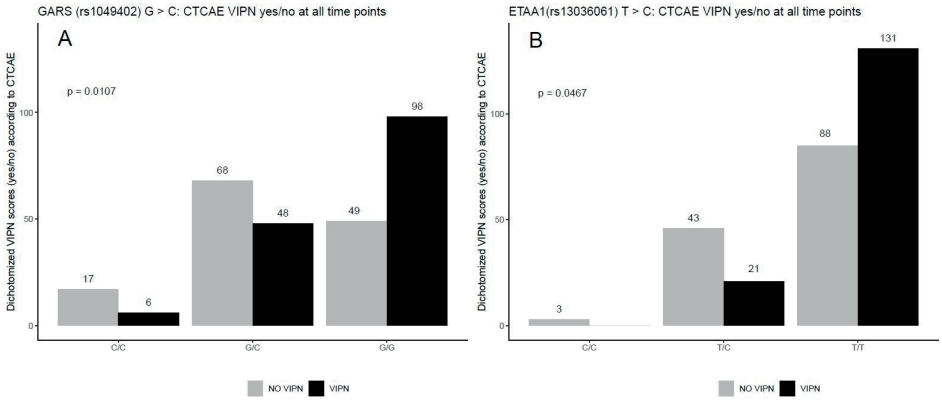

Figure 3. A. Association between a SNP in GARS and dichotomized (yes/no) VIPN scores according to the CTCAE score. B: Association between a SNP in ETAA1 and dichotomized (yes/no) VIPN scores according to the CTCAE score. VIPN measurements were performed 1–5 times in 85 patients. Every VIPN measurement per patient across the time points is shown. The number in the boxplot indicates the number of observations per genotype. A cut-off value of a CTCAE score of ≥2 was considered to be VIPN. The *p*-value was derived from mixed-effect logistic regression for repeated measures. For GARS, genotype was considered to be a categorical variable, whereas for ETAA1, genotype was considered to be an ordinal variable. ETAA1: Ewing's tumor-associated antigen 1.

Table 1. Patient characteristics of the included patients.

	Patients (Total Cohort) (*n* = 90)	Patients (PK Cohort) (*n* = 35)	Patients (DNA Cohort) (*n* = 85)
Age in years (mean, SD)	9.17 (5.15)	10.06 (5.60)	8.95 (5.00)
Ancestry (*n*, %)			
European	73 (81.11)	30 (85.71)	69 (81.18)
Non-European	17 (18.89)	5 (14.29)	16 (18.82)
Sex (*n*, %)			
Female	40 (44.44)	19 (54.29)	37 (43.53)
Male	50 (55.56)	16 (45.71)	48 (56.47)
Diagnosis (*n*, %)			
ALL	58 (64.44)	26 (74.29)	54 (63.53)
Hodgkin	18 (20.0)	6 (17.14)	18 (21.18)
Medulloblastoma	2 (2.22)	1 (2.86)	2 (2.35)
LGG	2 (2.22)	1 (2.86)	2 (2.35)
Wilms tumor	8 (8.89)	1 (2.86)	7 (8.24)
RMS	2 (2.22)	0 (0)	2 (2.25)
Mean (SD) cumulative VCR dose per m^2	7.41 (7.99)	13.25 (9.36)	13.91 (9.23)
Mean (SD) AUC ((ng·hr)/mL)	N.A.	41.78 (14.32)	N.A.
Mean (SD) VCR C_{max} (ng/mL)	N.A.	57.44 (31.82)	N.A.
Median (IQR) total CTCAE score	1.00 (0.00–2.00)	1.00 (0.00–3.00)	1.00 (0.00–2.00)
Median (IQR) total ped-mTNS score *	4.00 (1.00–8.00)	4.00 (1.00–8.25)	4.00 (1.00–8.50)
Patients with VIPN according to CTCAE (%)	40 (44.4)	16 (45.71)	40 (47.06)

* Total group was *n* = 66 (no. of patients aged ≥5 years), PK: pharmacokinetics, DNA: deoxyribonucleic acid, SD: standard deviation, ALL: acute lymphoblastic leukemia, LGG: low-grade glioma, RMS: rhabdomyosarcoma, VCR: vincristine, area under the concentration time curve, N.A.: not available, C_{max}: maximum plasma concentration of VCR, IQR: interquartile range, CTCAE: Common Terminology Criteria for Adverse Events, ped-mTNS: pediatric-modified Total Neuropathy Score, VIPN: vincristine-induced peripheral neuropathy.

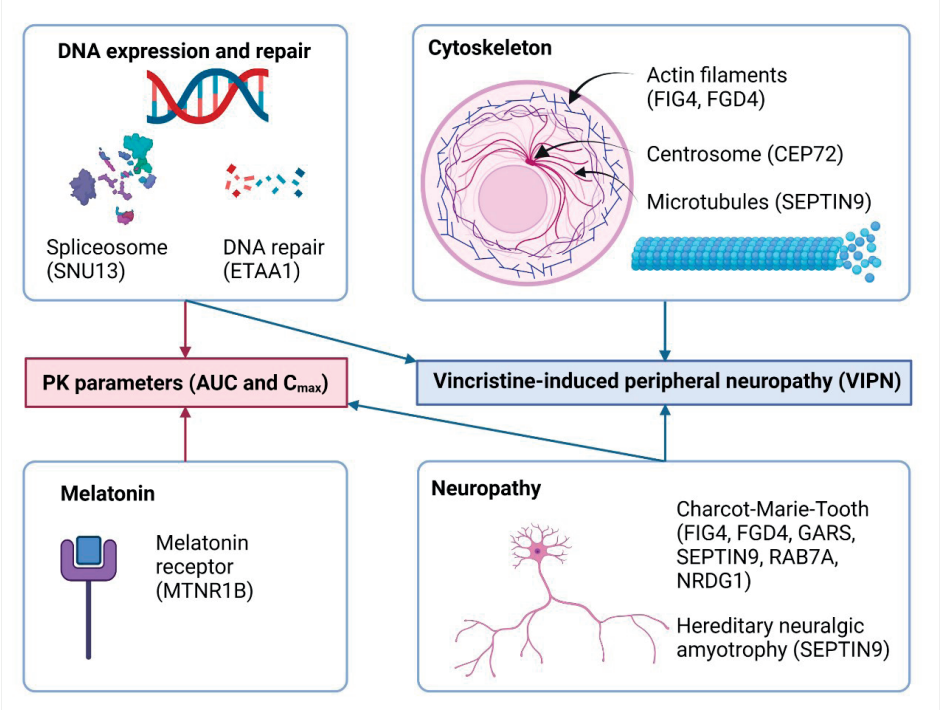

Figure 4. Schematic overview of the significant associations between genetic variations and VCR PK and VIPN. Figure created with Biorender.com.

Table 2. Associations between single-nucleotide polymorphisms (SNPs), VCR PK and VIPN.

Outcomes per SNP									Effect Size	
									Variant Heterozygous vs. Wild-type	Variant Homozygous vs. Wild-type
PK outcomes	SNP Gene	SNP RS-code	SNP Mutation	Consequence	Wild-Type *(n of Patients)*	Variant Heterozygous Genotype *(n of Patients)*	Variant Homozygous Genotype *(n of Patients)*	*p*-Value	Regression Coefficient (95% CI)	
AUC	MTNR1B *	rs8192552	G > A	Missense NMD transcript variant	G/G (28)	G/A (2)	A/A (0)	0.0032	20.42 (8.0–32.8)	N.A.
	RAB7A *	rs4548	C > T	Synonymous (intron)	C/C (28)	C/T (2)	T/T (0)	0.0011	23.54 (10.9–36.2)	N.A.
C_{max}	SNU13 *	rs6519270	A > C	Non-coding transcript variant (intron)	A/A (23)	A/C (8)	C/C (1)	0.0029	26.72 (7.4–46.0)	69.55 (23.0, 116.1)
VIPN Outcomes	SNP Gene	SNP RS-code	SNP Mutation	Consequence	Wild-type (*n of Patients)*	Variant Heterozygous Genotype *(n of Patients)*	Variant Homozygous Genotype *(n of Patients)*	*p*-Value	Ratio of Mean (95% CI)	
Total CTCAE	NDRG1 *	rs2272653	G > A	Splice region variant (intron)	G/G (34)	G/A (28)	A/A (19)	<0.0001	0.84 (0.73–0.96)	0.49 (0.40–0.60)
	GARS *	rs1049402	G > C	Missense transcription factor binding site variant	G/G (43)	G/C (32)	C/C (6)	0.0013	0.71 (0.62–0.82)	0.47 (0.34–0.64)

Table 2. Associations between single-nucleotide polymorphisms (SNPs), VCR PK and VIPN. (*continued*)

	Outcomes per SNP								Effect Size	
									Variant Heterozygous vs. Wild-type	Variant Homozygous vs. Wild-type
Total ped-mTNS	FIG4 *	rs9885672	T > C	Missense UTR variant of the 5′ UTR	T/T (40)	T/C (19)	T/T (0)	<0.0001	1.44 (1.30–1.59)	N.A.
	FIG4 *	rs10659	G > A	UTR variant of the 3′ UTR	G/G (53)	G/A (6)	G/G (0)	<0.0001	1.53 (1.34–1.75)	N.A.
	FGD4 *	rs12823621	G > A	Splice region variant (intron)	G/G (45)	G/A (13)	A/A (1)	<0.0001	1.43 (1.27–1.60)	1.88 (1.36–2.59)
	FGD4 *	rs73083501	C > T	NMD transcript variant (intron)	C/C (39)	C/T (18)	T/T (2)	<0.0001	0.86 (0.76–0.97)	0.46 (0.32–0.68)
	SEPTIN9*	rs11650934	C > G	UTR variant of the 5′ UTR	C/C (41)	C/G (17)	G/G (1)	<0.0001	0.62 (0.54–0.71)	0.81 (0.48–1.38)
	CEP72*	rs71585289	C > G	Upstream gene variant (intron)	C/C (27)	C/G (25)	G/G (6)	<0.0001	0.84 (0.75–0.93)	0.53 (0.43–0.66)
	ETAA1*	rs35777125	G > A	Non-coding transcript variant (intron)	G/G (43)	G/A (15)	A/A (1)	0.0007	0.91 (0.82–1.02)	0.36 (0.19–0.70)

Table 2. Associations between single-nucleotide polymorphisms (SNPs), VCR PK and VIPN. (*continued*)

Outcomes per SNP								Effect Size	
								Variant Heterozygous vs. Wild-type	Variant Homozygous vs. Wild-type
Dichotomized VIPN outcomes	SNP				Genotype (*n of patients*)	VIPN + (*n of observations*)	VIPN − (*n of observations*)	p-value	Odds ratio (95% CI)	
	Gene	RS-code	Mutation	Consequence						
VIPN (yes/no) according to CTCAE	GARS *	rs1049402	G > C	Missense transcription factor binding site variant	G/G (43)	98	49	0.0107	0.52 (0.28–0.99)	0.18 (0.03–0.92)
					G/C (32)	48	68			
					C/C (6)	6	17			
	ETAA1 **	rs35777125	G > A	Non-coding transcript variant (intron)	G/G (62)	131	88	0.0467	0.31 (0.16–0.57)	
					G/A (18)	21	43			
					A/A (1)	0	3			

SNP: single-nucleotide polymorphism, CI: confidence interval, NMD = nonsense-mediated decay, UTR = untranslated region. * *p*-value indicates statistical significance of overall genotype effect on the phenotype as described by the regression model. For the PK parameters mixed-effect linear models were fit, a random effect was included to account for intra-patient (repeated measure) correlation. For the total grade by CTCAE or ped-mTNS (an integer-valued phenotype), Poisson regression model was fit. For the dichotomized VIPN by CTCAE, mixed-effect logistic regression model was fit. Apart from one exception (see footnote below), genotype was regarded as a categorical variable.** Given the small number of homozygotes for the variant allele, OR was expressed as carriers of the variant allele (G/A and A/A) compared with homozygous wild-type (G/G).

RESULTS

Study Population

In total, 90 patients participated in the RCT, of whom 85 had sufficient DNA material available for analysis and thus were included in the DNA cohort in which the association between genetic variations and VIPN was described. For the CTCAE, 286 measurements were available in 85 patients with a median of four measurements per patient (interquartile range (IQR): 1–4). For the ped-mTNS, measurements were available in 59 patients with a median of three measurements per patient (IQR: 3–4). Furthermore, 35 out of 90 patients participated in the PK part of the trial (n = 70 occasions, 425 samples). These patients were included in the PK cohort in which the association between genetic variations and PK parameters was described. Patient characteristics are displayed in Table 1.

Genomic Analysis

The genotype data on a total of 767 SNPs were available and out of these, 263 SNPs had a minor allele frequency (MAF) of over 5%. After LD pruning ($r^2 \geq 0.30$), the number of SNPs was reduced to 98. In the total PK group, the median AUC was 39.78 (ng·hr)/mL and median C_{max} was 57.28 ng/mL. Furthermore, 44 out of 90 (49%) patients developed VIPN based on CTCAE and 55 out of 66 (patients ≥ 5 years of age; 83%) developed VIPN based on ped-mTNS. In total, 12 SNPs of ten genes were nominally significantly associated with any of our outcomes which all except two SNPs passed stringent correction for multiple testing ($p < 0.004$, FDR = 23%).

Three SNPs in three genes (Small Nuclear Ribonucleoprotein 13 (SNU13), RAS-related protein 7A (RAB7A), Melatonin Receptor 1B (MTNR1B)) were significantly associated with the PK of VCR (Figure 1). Patients with one or two minor alleles in these genes had higher plasma C_{max} or plasma AUC values (Table 2). The intronic SNP in SNU13 is an eQTL for SNU13 (Table S1). The estimated impact on protein function of the missense variant in MTNR1B was moderate (Table S1). None of the SNPs exhibited estimated splicing effects (Table S1).

Nine SNPs in seven genes were associated with total VIPN (Figures 2 and S1) and/or dichotomized VIPN scores (Figure 3), of which six and three SNPs were associated with lower and higher VIPN scores, respectively (Table 2). A missense variant located in the transcription factor binding site for Glycyl tRNA Synthetase (GARS) was associated with both total and dichotomized CTCAE scores. Similarly, an intronic SNP that is an eQTL for ETAA1 was associated with both total ped-mTNS score and dichotomized CTCAE score (Table S1). Two untranslated regions (UTR) variants in FIG4 Phosphoinotiside 5-Phos-

phatase (FIG4) were associated with VIPN, one of which is a missense variant with estimated moderate deleteriousness for protein function and one is an eQTL for FIG4 (Table S1). For FYVE, RhoGEF and PH Domain Containing 4 (FGD4), two SNPs were significantly associated with VIPN, of which one is an eQTL for FGD4 (Table S1). Finally, three intronic SNPs in N-Myc Downstream Regulated 1 (NRDG1), Septin 9 (SEPTIN9), and Centrosomal Protein 72 (CEP72) were associated with VIPN. As with the SNPs associated with the PK values, none of the SNPs exhibited estimated splicing effects (Table S1).

The results for all associations were similar in the multivariable analysis (Figure S2). A systematic overview of the function of the genes with SNPs that had significant associations with PK or VIPN is presented in Figure 4.

DISCUSSION

In this study, we have shown that genetic variants are associated with important VCR PK variables in pediatric oncology patients. We also replicated previously identified genetic associations involving VIPN and we have identified new genetic variants associated with VIPN. Since VIPN is a dose limiting toxicity, leading to impaired health-related quality of life in children[3], it is of utmost importance to identify patients at risk of developing VIPN and where possible to modify treatment dose to mitigate toxicity. However, such dose modifications should not result in low VCR exposure that could compromise anticancer effects of VCR. It is therefore important to not only understand the association between VIPN and genetic variants, but also VCR PK and genetic variants. Our study is, to our knowledge, the first to assess the association between PK of VCR and genetic variants in children with cancer.

Three SNPs in three genes were associated with the PK of VCR. First, we described a SNP that was an eQTL for SNU13, which is a highly conserved gene involved in pre-mRNA splicing as a component of the spliceosome[40]. Low expression of this gene is associated with increased sensitivity of primary leukemia cells to VCR, and concomitant use of SNU13 inhibitors and VCR both increases cytotoxicity of VCR and reduces VCR effects on neurons[41]. The reported SNP has not previously been described in relation to VIPN or PK of VCR. However, this specific eQTL variant may contribute to SNU13 expression variation and consequently affect the PK of VCR. Furthermore, a missense variant in MTNR1B was associated with the PK of VCR. MTNR1B is known for its role as coding for the melatonin receptor, but is also associated with other diseases such as type 2 diabetes[42,43]. Diouf et al. first described another SNP in MTNR1B in relation to VIPN[5]. Hypothetically, this association may be the result of PK differences between genotypes. The SNP found in this study has not been previously described in relation to VIPN or PK of VCR. Finally, a SNP in RAB7A was associated with the plasma AUC of VCR. RAB7A encodes for a protein that regulates vesicle traffic in the late endosomes and from the late endosomes to lysosomes. This SNP in RAB7A has been described in relation to CMT type 2[44]. In addition, this SNP was a C to T transversion, a variation that may be the result of oxidative stress[45,46]. Oxidative stress is a significant cause of DNA damage, not only in cancer cells, but also in germline cells[45,46].

Nine SNPs in seven genes were associated with VIPN, of which four genes are related to the cytoskeleton (Figure 4). VCR exerts its cytotoxic effect via the inhibition of mitosis, by acting on the cytoskeleton of the cell, establishing a biologically plausible links between genes involved in the cytoskeleton and VIPN[1]. First, we found a SNP in CEP72, a gene encoding for a centrosomal protein that is required for adequate chromosome

segregation. CEP72 in relation to VIPN was first described by Diouf et al.[5]. They found that a promotor variant in CEP72 was linked to VIPN in both children[47,48] and adults[49]. The role of this SNP was recently confirmed in a meta-analysis[18]. However, the impact of this SNP appears limited to the certain treatment phases, such as the continuation phase of ALL treatment, since the significant association was not replicated in the induction phase[50,51]. Of note, the SNP that we found in this study has not been previously described in relation to VIPN. It is not in LD with the SNP from Diouf et al.[5]. Nonetheless, we confirm the role of CEP72 in relation to VIPN in this study. Second, we found SNPs in FIG4 and FGD4, genes that are both involved in the regulation of the actin cytoskeleton and cell shape[52-55] and have been implicated in CMT type 4[56,57]. For FIG4, one SNP that was associated with an increased risk of VIPN has been previously described in adults with multiple myeloma as a risk factor for VIPN[58]. Furthermore, one of the SNPs in FGD4 is a C to T transversion, which may be the result of oxidative stress, as described above[45,46]. Thirdly, we found a SNP in SEPTIN9, a gene that encodes for a protein involved in cytokinesis and cell cycle control via the microtubules[59,60]. The gene has been implicated with hereditary neuralgic amyotrophy[59,60]. The reported SNP in SEPTIN9 has not been described in relation to VIPN previously.

In addition to the SNPs described above, SNPs in two more genes have previously been described in relation to CMT or hereditary neuropathies (Figure 4). One SNP in NRDG1, a gene encoding for an intracellular protein that can be induced under a wide variety of stress and cell growth conditions, is associated with CMT type[61,62]. Furthermore, one SNP in GARS, a gene that has been identified as a causative gene responsible for the clinical features of distal hereditary motor neuropathies type 4, was seen in patients suffering from CMT or sensory neuropathy[56,63-65]. Interestingly, opposed to their findings, the minor allele was associated with less neuropathy in our study population.

One SNP in a gene involved in DNA repair was associated with VIPN, namely a SNP in ETAA1 (Figure 4). The biological function of ETAA1 is not well described, but it appears to function as a DNA replication stress response protein and thus is important for genome stability[66,67]. Diouf et al. described another SNP in ETAA1 in relation to VIPN[5], although this association was not replicated in the adult population[68].

To investigate the association between genetic variants and VIPN, the method of VIPN measurement is an important consideration. Previously, this was mostly done via retrospective CTCAE assessment[2]. However, it was shown that up to 40% of children with VIPN are not identified using this method[69]. New VIPN assessment tools have been developed, such as the ped-mTNS, which performs better than those historically

used and are currently recommended for the measurement of VIPN in children[70,71]. In our study, we used specifically trained assessors for the prospective measurements of VIPN with two different tools, therefore identifying more children with VIPN symptoms than in previous studies. We thus used three different methods to identify children with VIPN, namely total CTCAE and ped-mTNS score and dichotomized CTCAE score. Of note, only two SNPs in GARS and ETAA1 had significant associations with two out of the three methods. As described above, the CTCAE and ped-mTNS measure symptoms of VIPN differently, which may explain this discrepancy. For further replication studies, it is important to take this into consideration. Moreover, it would be interesting to assess the effect of different dichotomization cut-off values, to study for example the effects of genetic variants on severe VIPN and any grade of VIPN compared to no grade of VIPN.

Our study has some limitations which should be considered. First, our sample size was small (n = 85) for assessing the association between genetic polymorphisms and VIPN with adequate power and more so for genetic factors associated with PK (n = 35). Nonetheless, our results replicated previously reported genetic polymorphisms, which seems to endorse those previous reported associations. However, due to the exploratory nature of this study, the newly identified genetic factors should be interpreted as preliminary and await external replication, since there was no independent replication cohort available. In addition, although the stringent FDR threshold to correct for multiple hypothesis testing was met for most of our findings, the FDR rate was still 23%, consistent with the possibility of a substantial number of false positive findings. Nonetheless, many of our findings involve genes previously linked to neuropathy, providing increased confidence in their association with VIPN.

The association between genetic factors and VIPN has frequently been studied. Many genetic factors appear to be associated with VIPN; however, as previously mentioned, results of the various studies are difficult to compare due to the diversity of the tools used to assess VIPN. This could be an explanation why many associations were only discovered in a single study and could not be confirmed in other studies. Although, it can also reflect the multifactorial nature of VIPN[2]. Unfortunately, this means that the single SNPs associated with VIPN have a small effect size, because the development of VIPN is dependent on so many factors. Individualized VCR dosing strategies based on single SNPs aiming to reduce VIPN is therefore probably not feasible. Similarly, upfront screening of patients who will receive VCR based on single SNPs will not identify those at highest risk of developing (severe) VIPN. It is thus of importance to relate relevant SNPs to each other, to establish a genetic risk score. To identify those relevant SNPs, future studies would ideally perform a whole genome strategy

in a sufficient sample size, corrected for relevant confounders such as cumulative VCR dosage, and with adequate prospective assessment of VIPN to study the association between genomic factors and VIPN. Furthermore, PK sampling should be part of this study to identify whether an identified SNP in a particular gene affects VIPN directly, or also affects VCR PK, since dose adaptation of VCR to prevent VIPN development should not result in VCR exposure below therapeutic efficacy. So far, no such study has been performed.

CONCLUSIONS

In conclusion, we identified and replicated several genetic associations between VCR PK and VIPN in children with cancer. The recognition that the occurrence of VIPN and VCR PK are associated with genetic polymorphisms in plausible genes provides insights that may prove useful in optimizing VCR treatment of children with cancer.

SUPPLEMENTARY MATERIALS:

Supplementary materials 1. Selection of the 48 candidate genes that were analyzed in relation to VCR PK or possible association with VIPN based on the function of the expressed gene and the SNPs included in the analysis.

Figure S1. Genetic variants significantly associated with VIPN according to the CTCAE and ped-mTNS.

Figure S2. The estimates of the coefficients with and without adjusting for covariates (disease, cumulative VCR dosage and ancestry).

Table S1. CADD en REVEL scores for all missense SNPs for predicted deleteriousness for protein function, delta score from SpliceAI for estimated impact on splicing and description of SNPs that are located in eQTL and their respective tissue types.

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CHildren treated with vincristine: A trial regarding Pharmacokinetics, DNA And Toxicity of targeted therapy In pediatric oncology patients. (CHAPATI)

M. E. van de Velde, A. Uittenboogaard, F Njuguna, T Vik, S Langat, GJL Kaspers

ABSTRACT

Objective Vincristine is among the most widely used and potentially effective chemotherapeutic agents in pediatric oncology patients. However, in black African children it is often sub optimally dosed due to genetic differences in the metabolism of vincristine. This study aims to optimize the dosing regimen of vincristine using therapeutic drug monitoring while carefully monitoring toxicity.

Design This will be a prospective cohort study. All children aged 2-14 years diagnosed with cancer and scheduled to receive vincristine can be included. After the administration of vincristine, blood samples will be taken. These will be shipped to and analyzed in the Netherlands to determine the vincristine concentration in each sample. Based on this, a dose advise will be given for subsequent vincristine administrations.

This cycle will be repeated in total 3 times. Toxicity will be monitored by determination of the bilirubin, by questionnaires and by physical examination to check for signs of peripheral neuropathy.

Results We aim to include 100 patients. The required dose to achieve a therapeutic vincristine level, will be compared to a historic cohort. The results will lead to an alteration in the dosing regimen of vincristine in all pediatric oncology patients in black African, thereby improving therapeutic efficacy and outcome of African pediatric oncology patients.

INTRODUCTION/LITERATURE REVIEW

Vincristine (VCR) is a vinca alkaloid which is among the earliest chemotherapeutic agents and has been used since the early 1960's[1]. It works by inhibiting the formation of microtubules in the mitotic spindle[2,3]. VCR is metabolized in the liver into M1, its active metabolite. Any cause of hepatic dysfunction, may alter the capability of the liver to metabolize VCR to M1, thereby leading to higher plasma levels of VCR[1]. Due to its affordability, wide availability, broad efficacy, the lack of myelosuppression and the fact that it is usually well tolerated, it is widely used among many pediatric oncology treatment protocols in low- and high-income countries[4]. The commonest side effect is VCR induced peripheral neuropathy (VIPN). This is a dose-limiting side effect causing a symmetric sensory-motor neuropathy[5-7]. However, there seems to be a racial difference in the development of VIPN, where Caucasians are more often affected than non-Caucasian patients[8-12]. Although toxicity is generally something to be avoided, the downside is that this low incidence of VIPN is associated with lower plasma levels of VCR and thereby also suboptimal therapeutic effectiveness of VCR[4]. This could be a contributing factor to the observation that non-Caucasian patients in general have a poorer outcome of treatment for several pediatric oncology diseases, such as non-Hodgkin's lymphoma, acute lymphoblastic leukemia and nephroblastoma[13,14].

Standardized dosing of VCR in Caucasian patients is 1.5-2 mg/m^2 with a maximum of 2mg. Increasing this dose has led to intolerable toxicity in these patients[10], however standard dosing nowadays in Kenya is 2mg/m^2 with a maximum dose of 2.5mg and this is generally well tolerated. It is thought that genetic determinants of VCR metabolism between Caucasian and non-Caucasian patients are responsible for this difference in pharmacokinetics and VIPN. One genetic variant that is especially well studied, is the CYP3A family of enzymes. High-expressers of the CYP3A5 genotype metabolize VCR up to five times more efficiently than low-expressers of CYP3A5[15]. Furthermore, there is a racial difference in the distribution of CP3A5 high-expressers and low-expressers, 60- 70% of African-Americans are high-expressers of CYP3A5 compared to only 18-30% in Caucasians [12,16]. In a cohort of Kenyan black children, 91% of children were CYP3A5 high-expressers[4]. The observation that black African patients experience less VIPN, have a worse therapeutic outcome and metabolize VCR quicker than Caucasian patients, led to our hypothesis that African patients have a sub therapeutic VCR treatment due to relative under dosing[4]. Therefore, the aim of the current study is to optimize VCR dosing in black Kenyan patients treated for a malignancy in the Moi Teaching and Referral Hospital (MTRH) by increasing the VCR dose, while carefully monitoring the VCR concentrations in the blood, toxicity in general and VIPN in particular. Furthermore, we would like to collect DNA samples to

link the VCR concentrations in the blood to (the identification of) genetic variants that influence the development and severity of VIPN.

JUSTIFICATION AND OBJECTIVES

Justification

Optimizing treatment by maximizing the use of VCR, which is an already available treatment option, is a simple way to improve survival for children with cancer. The risk for an increased rate of VIPN can be reduced by careful monitoring and by dose adaptations based on unacceptable levels of VIPN. Improving dosing strategies based on individual patient characteristics, such as race, can improve anticancer treatment regimens for African patients in Kenya and internationally.

Objectives

Broad Objectives:

1. To determine the optimal vincristine dose among children receiving chemotherapy at MTRH.

Specific Objectives :

1. To evaluate development and severity of peripheral neuropathy and hyperbilirubinemia in optimized vincristine doses in African pediatric oncology patients in MTRH.
2. To study genetic variants that are associated with vincristine metabolism and development and severity of peripheral neuropathy.

MATERIAL AND METHODS

Setting

The study will be carried out at Moi Teaching and Referral Hospital (MTRH) which is situated in the Western part of Kenya. The estimated catchment population MTRH is serving is between 18-20 million people. This is 40-45% of all inhabitants of Kenya.

MTRH has a bed capacity of 800 patients. The pediatric inpatient service is housed within the Shoe4Africa building with the pediatric oncology ward situated in the first floor of the building. There are two Pediatricians who are dedicated to the unit and who are leading the team that offers the clinical care. Pediatric Registrars rotate in the unit in about every three months. Other personnel attached to the unit include medical officer, clinical officer, nutritionist, nurses, physical and occupational therapists and social workers.

Study Design

This will be a prospective cohort study.

In- and exclusion criteria

Inclusion criteria:

- Black patients aged 2-14 years with a malignancy for which they are scheduled to receive a minimum of four VCR administrations as part of their treatment protocol:
    - Children who are treated for acute lymphoblastic leukemia;
    - Children who are treated for non-Hodgkin's lymphoma;
    - Children who are treated for rhabdomyosarcoma;
    - Children who are treated for neuroblastoma;
    - Children who are treated for nephroblastoma;
    - Children who are treated for retinoblastoma;
- Written informed consent

Exclusion criteria:

- Severe malnutrition (Z score ≤2 SD of weight for height)
- Total bilirubin >3 times upper limit of normal
- Pre-existent severe mental retardation e.g. Down syndrome

Study Execution

Patients will be identified as new admissions at the pediatric oncology ward of the Shoe4Africa building of the MTRH during morning rounds. After confirmation of a pediatric oncology diagnosis, for which a child is scheduled to receive several VCR

administrations, a member of the research team will contact the patient and their parent(s)/guardian(s) to ask informed consent. Furthermore, patients who are already being treated for a malignancy with vincristine but are scheduled to receive more vincristine are eligible for inclusion as well, if they do not have VIPN. They will be asked during outpatient or inpatient clinic visits by the research team for informed consent.

Before the next administration of VCR, a baseline measurement to assess the possible presence of peripheral neuropathy will be done. This measurement is based on the Common Toxicity Criteria of Adverse Events (CTCAE version 4.03). The CTCAE consists of over 330 items scoring adverse events of cancer treatment in 26 different categories. Possible grades range from zero (no symptoms) and three (severe complaints) or, if applicable, five (death)[17]. The CTCAE items used for the assessment of VIPN are constipation, peripheral sensory neuropathy, peripheral motor neuropathy and neuralgia. The maximum CTCAE score of these items is 18. Participants with a total score of two or higher are considered to have VIPN[8].

Patients are asked if they experience problems as described in the concerning CTCAE items. For the item peripheral sensory neuropathy, additionally deep tendon reflexes (DTR's) of the patella and Achilles will be assessed. Besides the CTCAE, VIPN will be tested by use of the pediatric modified total neuropathy scale (ped-mTNS). This is a validated tool for the assessment of neuropathy in children[18]. It consists of a standardized questionnaire and physical examination. It is a non-invasive tool to assess neuropathy in children aged 5-18 years and takes approximately 15 minutes to assess. Patients with a ped-mTNS score of 5 or higher are considered to have VIPN. In Appendix A and B in Chapter 3 the ped-mTNS can be found.

Besides VIPN monitoring, toxicity will be monitored by studying peripheral blood results which are taken for regular assessment during treatment.

Furthermore, before the next VCR administration, total bilirubin will be assessed. The Z score for malnutrition (weight for height) will be calculated. If the Z score is ≤ 2 SD or if the total bilirubin is > 3 times the upper limit of normal, the child is excluded from study participation.

After all baseline measurements are performed and the child is scheduled to receive the next VCR administration, the child will start with a regular VCR dose of 2.0 mg/m^2 with a maximum dose of 2.5 mg. After administration, blood will be obtained at t=15 min, t=30 min and t=75 min. This will be done by a finger prick, after which blood is

collected on a dried blood spot (DBS) paper form. After collection, the blood samples will immediately be shipped to Antoni van Leeuwenhoek (AvL) Hospital in Amsterdam, the Netherlands, where they will be analyzed once weekly. Collection and analysis of the sample will be similar to the method previously described by Skiles et al.[4].

Concentration of VCR in these collected samples will be compared with a historical cohort of VCR blood in Caucasian patients. When the studied DBS samples are below -20% of the expected median value of VCR in the historical Caucasian cohort, the dose will be increased by 20% (without dose capping). If the studied samples are above +20% of the target AUC of VCR in the historic cohort, the dose will be decreased by 20%. If the studied samples are between -20% or +20% of the expected median value of VCR in the cohort, the dose will be maintained in the following VCR administrations. The dose of VCR will only be increased in case of absent severe VIPN or any other severe side effect, and when VCR concentration is <20% below expected median value. At the first scheduled administration of VCR after this dose adaptation, DBS samples will be taken once more, and analyzed after which a possible second dose adaptation will be given, similar to the first dose adaptation. This second dose adaptation is followed by a possible third dose adaptation without dose capping and then a final fourth round of DBS sampling and analysis, but no further dose will be increased after this final fourth round of sampling. For the scheduled dose adaptations per treatment protocol, see Appendix II. Therefore, participants of this study have a maximum total increase of 73% of the dose of VCR than the starting dose, going up to 3,46 mg/m^2. See figure 1 for an overview of the study measurements. During the time of analysis of the samples (maximum 3 weeks), previously used VCR dosed is maintained, unless unacceptable VIPN, hyperbilirubinemia or severe malnutrition develops, then VCR dose is reduced by 50%. If these complications persist, VCR is omitted. Just before the adapted doses are administered, initial exams of VIPN assessment, bilirubin level and calculation of Z score of weight for height will be repeated.

Furthermore, before the 1st adapted dose is administered, a blood collection tube for DNA is taken during regular blood controls. These DNA samples will be analyzed once data collection is completed to link genetic factors to pharmacokinetic parameters of VCR and to VIPN. If there is excessive toxicity (defined as a ped-mTNS sore 9 or higher, a sum score of the CTCAE of 5 or higher or an item score of the CTCAE of 3 or higher), extreme malnutrition (defined as a Z score of weight for height <2 SD) or a hyperbilirubinemia (total bilirubin >3 times upper limit of normal), at any of these time- points, the planned VCR dose will be reduced by 50% despite of the dosing advice of VCR based on the studied samples.

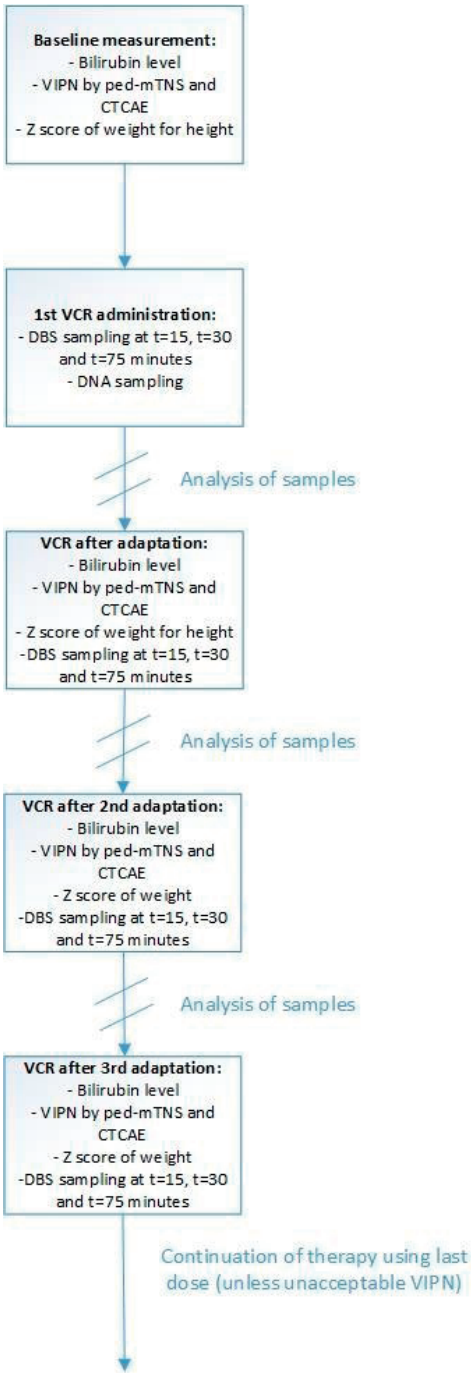

Figure 1. Study design and measurements
VIPN: vincristine induced peripheral neuropathy, ped-mTNS: pediatric modified total neuropathy scale, VCR: vincristine, DBS: dried blood spot

Due to the known interaction between VCR and azole antifungals, throughout this trial no concurrent use of VCR and azole antifungals will be administered, since this can lead to increased VCR toxicity [19].

This study will start with a feasibility study, in which 15 children aged 5 years or older are included. In summary, the feasibility study is similar to the CHAPATI study. The main difference lies in the collection of both dried blood spot (DBS) samples and plasma samples. The outline of the feasibility study is as follows: After first dosage administration, 3 fingerprick (DBS) will be taken at T=15, T=30 and T=75 minutes, as planned. Furthermore, we aim to collect 3 venous samples at T=15, T=30 and T=75 minutes as well. The venous samples will be retrieved from the same iv cannula as used for vincristine administration, to minimize unnecessary invasive procedures. Only children of 5 years or older are included in this feasibility study; a larger iv cannula can often be placed in older children, which increases the possibility of collecting 3 venous samples from the same iv cannula. However, if this is not possible, we will place maximum one extra iv cannula. Moreover, venous samples will only be collected from an iv cannula that is not needed for further medication or fluid administration, to prevent the need of placement of another iv cannula. The venous samples will be centrifuged to obtain plasma. The fingerprick DBS and plasma samples will immediately be shipped to the laboratory of the AvL in the Netherlands. The vincristine concentrations will be determined in both the DBS and plasma samples. The vincristine concentrations will be compared to a historic reference cohort. A dosage advice will follow based on the vincristine concentrations: if concentrations are below 20% of expected median value, a dosage increase of 20% is advised. If concentrations are above 20% of expected median value, a dosage decrease of 20% is advised. In case of any other value, no dosage adjustment is advised. This dosage advise will be provided within two weeks of sample collection. Regardless of whether a dosage adjustment was advised and/or given, another 3 DBS and 3 venous samples will be collected after the next adjusted vincristine dosage (similar as described above). Maximum 1 dosage adjustment can take place per included child in the feasibility study.

Statistics

A formal power analysis is not possible for this study due to the fact that it is currently unknown how many African children will have an expected median value outside the +/-20% target range of VCR. Therefore, the standard deviation of this number is also unknown and it is not possible to perform a power analysis. However, based on the occurrence of childhood cancer within MTRH, we hope to include 100 patients if possible within one year. This would give us sufficient data to determine the required VCR dose for an expected median value within the target range of African children.

Baseline data will be described quantitatively. Continuous variables of patients will be presented as means with standard deviations (SD) if normally distributed and medians with interquartile ranges if data are skewed; categorical variables will be given in percentages. Missing data will be omitted from the calculation of descriptive statistics. Summaries of patients will be presented in tables and/or graphs.

The primary outcome of the study, the expected median value, is measured at several timepoints (baseline and up to 3 times after dose adaptation). In order to estimate the effect of the intervention, a linear mixed model analysis with three levels (repeated measurements, patients, diagnosis) will be used. By using a linear mixed model analysis, first the difference in general over time will be analyzed. Secondly, by including time in the model, the effect of dose adaptations over time will be analyzed. In both analysis corrections will be made for scores at baseline and other relevant covariates, such as age and gender.

Pharmacokinetic analysis will include volume of distribution and total body clearance. All analyses will be performed using a nonlinear mixed effects modeling program (NONMEM, version 7.3, ICON Development Solutions, Ellicott City, MD, USA). Standard errors for all parameters are calculated using the COVARIANCE option of NONMEM[19]. To establish any differences between patients within and outside of the target AUC of VCR and between patients with and without VIPN, we will compare the frequency of specific polymorphisms between these groups. For statistical analysis we will dichotomize the data (within/outside target AUC, VIPN yes/no, SNP+/-) and perform Chi-square analysis.

Ethical considerations

This is a minimal risk, prospective cohort study. There is a small chance that VIPN development and severity increases due to higher VCR levels, however this will be carefully monitored. Patients might experience mild discomfort due to fingerpricks and possibly discomfort from placing maximum one additional iv cannula. Furthermore, the assessment of VIPN by CTCAE and ped-mTNS takes time, however all these assessments are done while the patient is hospitalized. Patients may directly benefit of participating in this trial by improving the exposure of VCR, leading to more optimized treatment. Data collected will be stored in a locked office in a file cabinet, and electronic data will be stored in a secure password protected database, with access limited to study personnel. All documents will be stored by studycode. Consent forms were part of submission.

The feasibility study will serve multiple goals. First of all, it aims to test the logistics of shipment of samples from Kenya to the Netherlands. We will assess the (possible) effect of shipment on the measurement of samples before initiation of the CHAPATI trial. Second, both DBS and venous samples will be collected in the feasibility study when providing a dosage advice. This aims to both build a reference cohort of Kenyan patients and to establish the relationship between vincristine concentrations in whole blood (in DBS samples) and in plasma (in centrifuged venous samples) in the Kenyan population. This relationship has only been established in European (predominantly Caucasian) children and we expect that vincristine metabolism is different in Kenyan children. Therefore, we want to establish this relationship in the Kenyan population before initiation of the CHAPATI trial. This feasibility study will create such a Kenyan reference cohort with maximum benefit for the children in the feasibility study (who will still receive an individualized dosage advice), while taking minimum risk (since venous samples are taken as well and only one dosage advice is given). Plasma samples are normally used to measure vincristine concentrations and their reliability and validity have been tested thoroughly. DBS assays, on the other hand, are a novel and promising technique. It is valuable to guarantee the reliability and validity of DBS vincristine measurements with the venous samples before initiation of the CHAPATI trial, where we will only be using DBS samples. Lastly, the times of sample collection have been determined based on a Caucasian reference cohort. We want to test these times in Kenyan patients before initiation of the CHAPATI trial.

Study implications

Results from this study will possibly improve treatment outcome due to a better exposure of VCR. It will likely demonstrate that children are currently receiving sub-optimal dosing. This information might lead to a change in treatment of a wide range of cancers for black African children worldwide, by demonstrating that these children benefit from higher doses of VCR without severely increasing VIPN. Hopefully, this will be shown eventually in improved survival rates.

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APPENDIX

Table 1: treatment regimens with included scheduled vincristine dose adaptations

Treatment protocol	1st VCR PK measurements	1st VCR dose adaptation	2nd VCR dose adaptation	3rd VCR dose adaptation
ALL	Induction: 1st VCR (if necessary: at 2nd VCR)	Week 16 (if necessary: week 17)	week 30	week 42
NHL	Prephase: 1st VCR (if necessary: course 2)	Course 3 (if necessary: course 4)	Course 6 (if necessary: course 7)	If possible: course 8
RMS	Pre-operative: week 1 (if necessary: week 2)	Week 8 (if necessary: week 12)	Week 20 (if necessary: week 24)	Week 32 (if necessary: week 36)
Neuroblastoma	Pre-operative: week 1 (if necessary: week 2)	Week 8 (if necessary: week 12)	Week 20 (if necessary: week 24)	Week 32 (if necessary: week 36)
Nephroblastoma arm A	Week 1 (if necessary: week 2)	Week 8 (if necessary: week 9)		
Nephroblastoma arm B	Week 1 (if necessary: week 2)	Week 8 (if necessary: week 9)	Week 14 (if necessary: week 17)	Week 23 (if necessary: week 26)
Nephroblastoma arm C	Week 1 (if necessary: week 2)	Week 8 (if necessary: week 9)	Week 15 (if necessary: week 18)	Week 24 (if necessary: week 26)
Retinoblastoma	Cycle 1 (if necessary: cycle 2)	Cycle 4 (if necessary: cycle 5)		

VCR: vincristine; PK: pharmacokinetics; ALL: acute lymphoblastic leukemia; NHL: Non-Hodgkin's lymphoma; RMS: rhabdomyosarcoma;

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General discussion

Over the past decades, survival of children with cancer has greatly improved, which has led to a shift in research from only improving survival to also reduce toxicity of treatment. Since vincristine (VCR) is already used for decades, the problem of VCR-induced peripheral neuropathy (VIPN) has been extensively studied. These studies have demonstrated that 20-100% (depending on measurement tool) of children treated with VCR develop VIPN during treatment[1], with many of them still experiencing neuromuscular impairments more than 10 years after VCR cessation[2-4]. VIPN is characterized by symptoms such as weakness of lower limbs, areflexia, neuropathic pain, constipation, and sensory loss. Although the negative impact of these symptoms on health-related quality of life (HR-QoL) has been demonstrated in adults[5], information on this subject in children is lacking. Therefore, we studied to which extent VIPN also reduces HR-QoL in pediatric oncology patients currently under VCR treatment. A negative association between VIPN and HR-QoL would underline the importance of finding ways to reduce toxicity of VCR treatment. It is in this context that we investigated whether prolonging the administration duration of VCR will result in less VIPN. The rationale for doing so is that previous studies have indicated that higher peak-plasma VCR concentrations, which occur when VCR is administrated in a short period of time (i.e. several minutes), are associated with VIPN[6]. To adequately study this, we first had to determine which are the best tools available to assess VIPN in pediatric patients. For this purpose, we studied the construct validity and reliability of the Dutch version of the pediatric modified total neuropathy score (ped-mTNS). The original version of the ped-mTNS, which is in English, is thought to be the best currently available tool for the assessment of VIPN in children[7,8]. Furthermore, in our effort to disentangle the multifactorial working mechanism of VIPN, we studied to which degree pharmacokinetics (PK) and genetic factors are associated with VIPN development.

ASSESSMENT OF VINCRISTINE-INDUCED PERIPHERAL NEUROPATHY IN CHILDREN WITH CANCER

In **Chapter 3**, we present our results regarding the validity and reliability of the Dutch version of the ped-mTNS. We have validated the findings as previously published by Gilchrist et al. regarding the construct validity of the original version of the ped-mTNS[9]. The availability of the ped-mTNS in Dutch enables the use of a more standardized assessment tool of VIPN among children in different studies, thereby improving the comparability between study results. The lack of a golden standard in the assessment of VIPN in children currently negatively affects the global research on VIPN. The variety of measurement tools assessing various study outcomes, both

prospective and retrospective, with each tool having a different sensitivity for detecting VIPN[9], makes it hard to compare different studies investigating VIPN. Furthermore, none of the currently used tools have been used in study groups that are large enough to properly assess inter- and intra-rater reliability. Good reliability is imperative for a measurement tool to be used as a golden standard outcome. Also, the content validity of used measurement tools (i.e. the degree to which the tool measures all the aspects of VIPN in terms of relevance and comprehensibility) should be assessed. As suggested by Lavoie Smith et al.[8] ideally this tool should be a patient-reported outcome measure (PROM) specifically developed for assessing VIPN in children. A patient-reported outcome is directly reported by the patient without interpretation of the patient's response by a clinician or anyone else and pertains to the patient's health, quality of life, or functional status associated with health care or treatment[10]. They reflect components important to the patient and may include patient reports of symptoms and other indices such as quality of life. The PROM is the measurement tool of the patient-reported outcome. Recently a PROM for VIPN in children was published: Pediatric Chemotherapy-Induced Neuropathy (P-CIN), but this tool is not yet validated in a large enough sample size[11]. The difficulty in developing an adequate PROM for VIPN in children lies in the fact that symptoms of VIPN, such as numbness or tingling, are difficult to describe or express by children. Children who can read can quantify these symptoms using simple words or by execution of easy assessments. However, for younger children (i.e. those who cannot read or properly verbally express their complaints) a tool should be used that can objectively quantify these symptoms, whereas in older children a more extensive tool should be used. Furthermore, preferably this PROM should be non-invasive and not require specially trained assessors, making it feasible for routine assessment of VIPN during chemotherapeutic treatment. To this end, it is recommended that an age-dependent PROM is being developed, which objectively assesses VIPN with regard to the developmental age of a child. Although it is less reliable, in the age-group 2-4 years this can only be done by including a (parent) proxy assessment as well. Moreover, if a PROM is available, it can be included as a routine assessment during treatment, outside of the scope of research, making physicians and others involved in the treatment of pediatric oncology patients more aware of the occurrence of VIPN in a patient, which could require medical intervention such as laxatives or use of analgesics.

Finally, retrospective chart-based review, which has frequently been used in the past to assess VIPN[1], should be discouraged as this is an inadequate method to assess VIPN, as the absence of VIPN in a medical chart does not rule out that a patient still experiences VIPN to some degree.

The difficulty of assessing VIPN in pediatrics and the lack of a reliable measurement tool is not unique to VIPN. This is a problem for several studies regarding adverse drug reactions in pediatric oncology. For instance, the CTCAE is used for the management of anticancer therapies and in clinical trials to provide standardization and consistency in the definition of treatment toxicity. However, the described adverse events per item are often not age-appropriate and therefore cannot always be used in pediatric oncology. In the CTCAE version 4.03 of June 24 2010, it was noticed that the description of 26 adverse effects items should be adjusted for use in children and 21 effects were missing for this population[12]. Unfortunately, these were not appropriately adapted in follow-up versions of the CTCAE[13].

EFFECT OF VINCRISTINE-INDUCED PERIPHERAL NEUROPATHY ON HEALTH-RELATED QUALITY OF LIFE IN CHILDREN WITH CANCER

In **Chapter 4** we describe the association between health-related quality of life (HR-QoL) and VIPN. Studies investigating the relation between HR-QoL and VIPN in children are scarce. Moreover, the previous studies that are available on HR-QoL among survivors of childhood cancer who suffer from VIPN, were conducted more than four years after cessation of VCR treatment[14,15]. The study in chapter four is the study that investigated the effect of VIPN on HR-QoL in children currently being treated for cancer.

We show that children with VIPN had a significantly lower HR-QoL on almost all domains (physical functioning, social functioning, emotional functioning, school functioning and pain), irrespective of method of assessment of VIPN (Common Terminology Criteria of Adverse Events (CTCAE) or ped-mTNS) and irrespective of self- or proxy-assessment. This study highlights the debilitating effect of VIPN. Children suffering from VIPN are at risk of performing not as well in school as other children with cancer who do not have VIPN. Hence, this study shows the importance for future research to investigate how we can optimize VCR therapy and reduce the risk of developing VIPN.

FACTORS CONTRIBUTING TO THE DEVELOPMENT OF VINCRISTINE-INDUCED PERIPHERAL NEUROPATHY IN CHILDREN

In **Chapter 2** we systematically reviewed the available literature on contributing factors, both patient-related and VCR-related, on VIPN in children with cancer. This

provided us with a good model to study the most important contributing factors of VIPN, and therefore possibilities to reduce VIPN. The model is presented in Figure 1 of Chapter 2.

Duration of administration of vincristine

Ultimately, our goal is to reduce the development and severity of VIPN in children with cancer. To this end **Chapter 5** presents the results of an intervention study that investigated the effect of administration duration on VIPN development in pediatric oncology patients. We conducted a randomized controlled clinical trial (RCT) comparing intravenous push-injections (1-5 minutes) with intravenous one-hour infusions of VCR. Overall, we did not find a statistically significant difference between the two groups regarding the occurrence or severity of VIPN. Interestingly, however, children who received concomitant azole antifungals together with VCR had significantly less severe VIPN when VCR was administered in one-hour. This could be due to the competitive drug-drug interaction between VCR and azole antifungals, which are both metabolized in the liver by CYP3A enzymes[16-19]. This interaction leads to slower metabolization of VCR into M1, the active metabolite of VCR, which could be more pronounced when the (peak) concentration of VCR is higher, i.e. when VCR is administered as a push injection. In other words, one-hour administration is preferred when co-medication is used with similar pharmacokinetic characteristics as azole antifungals or when drug-drug interactions are likely to occur.

Pharmacokinetics of VCR

The PK of VCR is dependent on multiple factors. First of all, the dose of VCR influences PK, and, as discussed in the aforementioned paragraph, so does concomitant drug use. However, the effect of certain other variables on PK of VCR is uncertain. Some studies showed an age-dependent relationship with clearance (Cl)[20], whereas some did not[21-23]. There was also no reported effect of sex on PK[20,21,23]. Moreover, there is a large inter-patient and intra-patient variability with regards to PK[20-22,24,25]. In **Chapter 6** we present the results of the PK outcomes related to administration duration (push injection (1-5 minutes) vs one-hour infusion) and the relation between PK and VIPN. This study confirmed our hypothesis that the maximum concentration (C_{max}) of VCR is indeed higher in the push group than in the one-hour group. In addition, we demonstrated that intercompartmental clearance (IC-Cl) and volume of the peripheral compartment (V_2) are significantly higher in the push group. Furthermore, it was shown that IC-Cl was significantly higher in patients with VIPN. This supports our hypothesis that longer lasting administration times of VCR can result in reduced levels of VIPN.

PK of children treated with VCR is also influenced by genetic factors. In **Chapter 7** we describe several genetic pathways associated with PK. We identified Melatonin Receptor 1B (MTNR1), RAS-related protein 7A (RAB7A) and Small Nuclear Ribonucleoprotein 13 (SNU13) to be associated with AUC or C_{max}. Recently, SNU13 expression was shown to be associated with increased sensitivity to VCR and VIPN development[26]. The association between C_{max} of VCR and VIPN offers a plausible working mechanism for increased VCR sensitivity in these cells. These associations provide insight on working mechanisms that can be used to find ways to reduce VIPN in children with cancer.

Genetic factors associated with vincristine-induced peripheral neuropathy

The association between genetic factors and VIPN has frequently been studied[1,27]. Many genetic factors appear to be associated with VIPN, however, as previously mentioned, results of the various studies are difficult to compare due to the diversity of the tools used to asses VIPN and heterogeneous study populations. This could be an explanation why many associations were only discovered in a single study and could not be confirmed in other studies[1]. In **Chapter 7** we describe the results of a genomic association study in which we studied 94 pre-identified genes and their relation to VIPN. We were able to validate the associations between a previously studied SNPs and VIPN: SNP rs1049402 in the Glycyl tRNA Synthetase (GARS) gene[28]. Furthermore, we identified several unknown SNPs in previously identified genes related to VIPN: rs35777125 (in Ewing's Tumor-Associated Antigen 1 (ETAA1))[29] and rs71585289 in Centrosomal Protein 72 (CEP72)[29]. Finally we found several SNPs in previously unknown genes related to VIPN: rs2272653 in N-Myc Downstream Regulated 1 (NRDG1), rs12823621 and rs73083501 in FYVE, RhoGEF And PH Domain Containing 4 (FGD4), rs10659 and rs9885672 in FIG4 Phosphoinositide 5-Phosphatase (FIG4) and finally rs11650934 in SEPTIN9.

All in all, these results show that many SNP's, from many different genes, seem to be associated with VIPN. They can be related to: 1) an altered sensitivity of neurons to the damaging effect of VCR; 2) to intracellular processes which are affected by VCR (such as microtubule formation); 3) to the metabolization of VCR (such as SNPs that enable a faster metabolization of VCR into M1, the main metabolite of VCR); or to 5) PK of VCR (which is dependent on many factors such as transporter proteins and receptor and proteins necessary for transport of VCR into the cell and for intracellular transport, amongst others). Unfortunately, this means that a single SNP associated with VIPN usually has a small effect size, because the development of VIPN is dependent on so many factors. It is difficult to adapt VCR treatment based on a single SNP to reduce VIPN. This also means it is difficult to identify patients upfront who

are at risk of developing (severe) VIPN, since screening on a single SNP would have little impact. Therefore, research effort should be put into the development of a multi-genic/multi-SNP based risk stratification model. In such a model, the effect of multiple SNP's/genetic aberrations are put together, ideally also combined with clinical characteristics, to provide an estimated risk of VIPN development after treatment. This is called a polygenic risk score. Based on this risk score, an individualized dosing regimen should be used to reduce the risk of VIPN development in those identified as high-risk patients.

STRENGTH AND LIMITATIONS

One of the strengths of this thesis is the multidimensional approach in how we have studied VIPN during oncologic treatment in children. Furthermore, we have longitudinally assessed all our outcomes. We have used two different measurement tools and specifically trained our assessors to measure VIPN. Moreover, we performed the first intervention study designed as a randomized controlled clinical trial that aimed to reduce the development and severity of VIPN in children, by prolonging the time of administration of VCR to one hour.

The studies conducted as part of this thesis have raised awareness to the problem of VIPN in children. Nowadays, a lot of research effort is being put in new drug discoveries, while optimization of treatment from older drugs is often overlooked. Especially in drugs who have been on the markets for several decades. In children this is even more problematic, because almost all older drugs have never been specifically tested in children and are used off-label. Therefore, the European Union (EU) adopted a law; the Paediatric Regulation [Regulation (EC) No 1901/2006 of the European Parliament and of the Council] which entered into force in January 2007. This law specifically aims to improve public health for children through increasing research, information and availability of medicines. Furthermore, since July 2008 it is mandatory to have a Paediatric Investigation Plan (PIP) for the validation of a marketing authorization application for adult and pediatric medicines currently not authorized in the EU (article 7 products). This means that newly developed drugs need to be specifically tested for efficacy and safety in specific doses in children before they are allowed to be used in clinical practice by children. However, this also means that most drugs who were introduced before 2007, such as VCR, were not specifically tested in children and data generated in adults were extrapolated for use in children, which increases the risk of dosing errors and adverse drug reactions[30-32]. As such, it is especially important to study drugs introduced before 2007 and assess possible adverse events, PK, and

optimum dosing strategies of these drugs in children. The funding program Goed Gebruik Geneesmiddelen (Good Use of Drugs) by NWO (the Dutch Organization for Scientific Research), who also funded the research in this thesis, aims to improve the effective and safe use of currently existing drugs, which contributes to the proper use of medicine in children.

This study also had some limitations. Since VCR is so widely used in so many treatment protocols in pediatric oncology, we decided not to focus on one specific disease type, since our outcomes could have an implication for all children treated with VCR. By doing so, however, we have introduced a heterogeneity in our study population, since children with different treatment plans, co-medications and varying cumulative VCR doses were included. However, this problem of multi-agent therapy is almost always encountered when studying a certain drug used for treatment of pediatric oncology patients. To ensure adequate comparison of the outcome of interest in the context of a multimodal therapy, a (RCT) in a large group of patients should be done, to divide possible confounders of the outcome of interest equally between the randomization arms. Furthermore, adequate statistical testing that can incorporate correction of certain confounders should be used. However, this all requires a large sample size, which is inherently difficult within the context of pediatric oncology research since pediatric cancer is a rare disease. This often results in pediatric oncology trials to be underpowered or difficult to complete within a reasonable timeframe[13]. Also our sample size was relatively small. Especially to reliably study PK and genetics the sample size should have been larger to unequivocally determine the influence of these parameters on VIPN. Therefore, these studies can be seen as hypothesis-generating rather than to state firm conclusions. This is also due to the fact that we used a targeted approach for our genetic analyses regarding the association with VIPN. Ideally, a whole genome strategy should be performed in a sufficient sample size and with adequate prospective assessment of VIPN to study the association between genomic factors and VIPN. So far, no such study has been performed. Diouf et al.[29] performed a genome-wide association study to identify CEP72 as a gene related to VIPN, but relied on retrospective chart-based CTCAE assessment to identify VIPN.

The studies presented in this thesis all had a follow-up time of one year. Although it is generally assumed that VIPN is a sub-acute condition that diminishes within several months after treatment cessation, studies have shown that symptoms and consequences of VIPN can still persist into adulthood of survivors of childhood cancer[2,3,14]. Therefore, our follow-up time might have been too short, especially to study the effect of administration duration on the development and severity of VIPN. Furthermore, even though the assessors were generally unaware of the randomization status of

the patients regarding administration duration, they were not formerly blinded. This could have introduced bias into our results.

GENERAL CONCLUSIONS AND FUTURE PERSPECTIVES

General conclusions

This thesis has shown that VIPN in children is a multifactorial toxicity, affected by ancestry, PK, dose and administration duration, co-medication, and genetic factors. Furthermore, we have shown the effect of VIPN on HR-QoL of children currently treated with VCR. We were the first to demonstrate that children with VIPN report lower HR-QoL than children with cancer without VIPN. Also, we demonstrated that children who are concurrently treated with azole antifungals and VCR, experience less VIPN when VCR is administered in one-hour instead of a push injection. Theoretically, one-hour infusions could be beneficial over push injections due to the fact that it leads to a lower C_{max} and lower IC-Cl, which showed an association with VIPN. Furthermore, the results of our studies have demonstrated that VIPN is associated with genetic factors such as SNPs in ETAA1, CEP72, NRDG1, FGD4 and FIG4 and PK of VCR is associated with MTNR1B, RAB7A and SNU13. Finally, currently there is no golden standard with respect to assessment tools of VIPN. There are some tools that could be used as golden standard if their psychometric qualities appear to be adequate. In our study we demonstrated that the psychometric qualities of the Dutch translated version of the ped-mTNS, which can be used in children aged ≥5 years, were sufficient and similar to the original English version of the ped-mTNS.

Future perspectives

New strategies should be developed to reduce VIPN in children, while maintaining adequate VCR treatment. To this end, each of the contributing factors described in this thesis should be taken into account. Pivotal in the prevention of VIPN in children is the understanding that there is no such thing as "one size fits all". In the future, VCR treatment should be used on a more individualized basis. To optimize this a number of factors should be taken into account, such as genomic features and a patient's individual PK profile. Currently, the St. Jude Children's Research Hospital Total XVII treatment protocol[33] is open for inclusion. As part of this study, children are screened upfront for the CEP72 rs904627 T/T high-risk genotype for VIPN[29]. If these patients are homozygous for the T/T genotype, they are randomized to receive reduced dose of VCR of 1.0 or the conventional 1.5 mg/m^2 starting at Continuation week 1. Children with a rs904627 C/T or CC genotype are randomized to receive 2mg/m^2 per dose (instead of 1.5 mg/m^2)[33]. Furthermore, recently Verma et al.[34] used a metabolomics

approach to identify metabolites that can predict VIPN development in children at day 8, 29 and at 6 months after start of treatment. With these identified metabolites, they developed a free user-friendly tool, VIPNp, for physicians to use their model to identify patients upfront that are at risk of developing VIPN during treatment.

To optimize individualized VCR treatment using a patient's individual PK, preparations for the start of the CHAPATI trial (*"CHildren treated with vincristine: A trial regarding Pharmacokinetics, DNA And Toxicity of targeted therapy In pediatric oncology patients")*, of which the study protocol is described in **Chapter 8**, are currently ongoing. In short, in this study, we investigate the impact of ancestry and PK on VCR exposure, thereby studying if it is beneficial to increase the dose of VCR in Kenyan children. Previously it was shown that African(-American) children experience less VIPN compared to Caucasian children[1,29,35]. Moreover, studies have also demonstrated that non-Caucasian patients in general have a poorer outcome of treatment for several pediatric oncology diseases[36,37]. This could be related to the fact that African children have a lower exposure to VCR using a standardized dosing regimen, because of the fact that many of these children are so-called rapid metabolizers[38]. Moreover, African children can tolerate a dose of 2 mg/m^2 with a maximum of 2.5 mg[38], whereas this dose in a cohort of mainly Caucasian has led to premature study termination due to intolerable VIPN[29]. This has resulted in the hypothesis that by using the current dose of 2 mg/m^2 with a maximum of 2.5 mg, Kenyan children have a lower area under the concentration time curve (AUC) compared to a historical cohort of mainly Caucasian children. This cohort consists of pooled datasets, among which is the data presented in Chapter 6. The CHAPATI trial is set up as follows: after a VCR administration blood samples from the patient will be collected at three time-points. Of these samples, the VCR concentration is determined after which the AUC is calculated. This AUC will be compared to values measured in a historical reference cohort of pediatric oncology patients. If the AUC is below the lower cut-off value, the subsequent VCR dose will be increased by 20%. If the dose is similar to the AUC in the previous cohort, the dose stays the same or if the dose is above a certain higher cutoff value, the subsequent dose will be decreased. This process (VCR administration – sampling – determination of VCR concentration – calculation of AUC – comparison with historical cohort – dose adaptation) is, whenever possible (i.e. sufficient VCR administrations according to the treatment protocol), repeated up to three times. Furthermore, there will be careful monitoring of VIPN. When there is excessive VIPN, the subsequent dose will be lowered irrespective of AUC.

Obviously, individualized treatment is not only advocated for VCR, but for many other drugs as well. In general, studies investigating these individualized treatment proto-

cols have two perspectives: from the cancer itself and from the patient who receives the drugs. For specific tumors it is advocated to identify risk-groups requiring more or less intensive treatment. These risk groups are derived from genetic mutational research or cytogenetics which have shown to alter the chance of survival of a specific tumor compared to other tumor types. Also, specific treatment targets within a tumor are identified which can lead to drugs specifically designed for these treatment targets within a tumor. These drugs can only be used in tumor types that have these drugable targets. This type of precision medicine is currently used as treatment or in trials within pediatric oncology[39]. From the patient's perspective, individualization of treatment is mostly focusing on pharmacogenomics: genetic factors resulting in slower or faster metabolization of drugs, possibly leading to dose adaptations. This was mostly studied in thiopurine S-methyltransferase (TPMT). It was shown that based on the TPMT activity in an individual the dose of mercaptopurine can be adapted, leading to similar treatment outcomes with reduced toxicity[40]. However, all these tests and dose adaptations require additional testing and therefore increase costs. Especially the use of tumor based targeted therapy leads to an increase in costs for a small number of patients, since drugs can only be used in certain tumor types that are sensitive to these drugs. This leads to new ethical questions regarding acceptable costs to optimally treat a child with cancer. However, since many children survive cancer and will live long afterwards, treatment of childhood cancer is almost always cost-effective.

All these developments, the incorporation of pharmacogenomics and the use of PK, will result in a more individualized dosing regimen of VCR in children. Currently, this is already standard practice in the treatment with certain antibiotics, both in children and in adults[41]. All this was done (and will be done) with the purpose to reduce VIPN and other toxicities, while maximizing the therapeutic effectiveness of VCR in children with cancer, thereby resulting in equal or even higher cure rate, and better HR-QoL of children with cancer.

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10

Summary

In this thesis we study different aspects of vincristine (VCR)-induced peripheral neuropathy (VIPN) in children with cancer. Vincristine is a commonly used chemotherapeutic agent in pediatric oncology. Its most important side-effect is VIPN. This is a debilitating dose-limiting toxicity resulting in sensory symptoms, such as pain, numbness or tinglings and/or motor symptoms, such as foot drop or difficulty to walk. It can also result in autonomic symptoms such as constipation. Since VCR is frequently used in many pediatric oncology treatment protocols, many children suffer from this toxicity. With the studies presented in this thesis, we want to gain more insight into which children are at risk of developing VIPN, improve the way VIPN is assessed, understand more of the relationship between VCR pharmacokinetics (PK) and VIPN and between genetic factors and VIPN. Moreover, this thesis aims to explore ways to reduce VIPN in children by reporting and discussing the results of an intervention study. Finally, the consequences of VIPN with respect to perceived health-related quality of life (HR-QoL) are discussed in this thesis as well as a new intervention study that aims to optimize VCR treatment in a specific population.

Childhood cancer in its entirety is a rare disease. The incidence of cancer among adults differs from the incidence among children. In children, acute lymphoblastic leukemia is the most common type of cancer, whereas adults mainly have solid tumors, especially carcinomas. The latter are very rare in children. The outcomes of childhood cancer have greatly improved over the last decades, which has led to a shift in research focus from only improving survival to also improving the quality of survival. VCR is one of the oldest types of chemotherapy used in children and was already discovered in the 1960's. It is derived from the *Catharanthus roseus* plant. It works by inhibiting mitosis within the cell. VIPN is the result of a complex pathophysiology: VCR leads to alterations in the immune system resulting in neuro-inflammation. It also alters action potentials and excitability within the neurons. Furthermore, there are diminished sensory fibers in the neurons and finally, it alters the cellular metabolism, also resulting in altered excitability. There is no specific treatment for VCR, only supportive care. The working mechanism of VCR, the pathophysiology of VIPN, and the contributing factors related to VIPN are described in more detail in **Chapter 1**.

Chapter 2 presents an overview of the available literature on the methods to assess VIPN in children as well as all the contributing factors resulting in VIPN in a systematic review. Studied factors include patient characteristics, VCR dose, administration method, pharmacokinetics, and genetic factors. Furthermore, this review reports on currently available methods to assess VIPN in children. In total, twenty-eight studies were included. First, the different ways VIPN is assessed in the different studies, with their different psychometric qualities is discussed. This diversity of methods

of VIPN assessment seriously impairs comparability across study outcomes. Results indicate that Caucasian race, higher single VCR dose, older age and low VCR clearance negatively influence VIPN, although results regarding the latter two factors are rather conflicting. Moreover, genetic pathways influencing VIPN are identified. In this chapter a schematic overview of all possible contributing factors towards VIPN is presented. These factors as well as their interplay are important to determine the occurrence and severity of VIPN in children.

In **Chapter 3** the Dutch translation of a newly developed method to assess VIPN, called the pediatric-modified Total Neuropathy Score (ped-mTNS), is tested for its construct validity and reliability in Dutch pediatric oncology patients aged 5–18 years. Construct validity (primary aim) of the ped-mTNS is determined by testing hypotheses about expected correlation between scores of the ped-mTNS (range: 0–32) and the Common Terminology Criteria for Adverse Events (CTCAE) (range: 0–18). This is done by comparing patients and healthy controls and by comparing patients and controls regarding their total ped-mTNS scores and the proportion of children identified with VIPN. Inter-rater and intra-rater reliability and measurement error (secondary aims) are assessed in a subgroup of study participants. Among the 112 children (56 patients and 56 age- and gender-matched healthy controls) evaluated, correlation between CTCAE and ped-mTNS scores is as expected (moderate ($r = 0.60$)). Moreover, as expected, patients have significantly higher ped-mTNS scores and more frequent symptoms of VIPN compared with controls (both $p < .001$). Reliability is measured within the intra-rater group ($n = 10$) (intra-class correlation coefficient ($ICC_{agreement}$) was 0.64, standard error of measurement ($SEM_{agreement}$) was 2.92, and smallest detectable change ($SDC_{agreement}$) was 8.1) and within the inter-rater subgroup ($n = 10$) ($ICC_{agreement}$ was 0.63, $SEM_{agreement}$ was 3.7, and $SDC_{agreement}$ was 10.26). These results indicate insufficient reliability. In summary, the Dutch version of the ped-mTNS appears to have good construct validity for assessing VIPN in a Dutch pediatric oncology population, whereas reliability appears to be insufficient and measurement error high. All in all, standardization of VIPN assessment in children needs to be improved. To this end, future research aimed at evaluating and further optimizing the psychometric characteristics of the ped-mTNS is needed.

In **Chapter 4** the effect of VIPN on health-related quality of life (HR-QoL) during pediatric cancer treatment is described. VIPN was measured at baseline and 1–5 times during treatment using CTCAE and ped-mTNS. Assessment of HR-QoL was done with self-and proxy assessment of the Cancer and Generic module of the Pediatric Cancer Quality of Life Inventory™ (PedsQL). In total, $n = 86$ children were included. HR-QoL of children with VIPN ($n = 67$, 76%) is significantly lower in comparison with children

without VIPN: estimated Total score of PedsQL Generic (proxy) 84.57; β = −8.96 and 95% confidence interval (CI) −14.48 to −3.43; p = 0.002, estimated PedsQL Generic Total score (self-reported): 85.16, β = −8.38 (95% CI: −13.76 to −3.00); p = 0.003. Similar results are found in the Pain and Hurt domain of the PedsQL Cancer (estimated score (proxy): 85.28, β = −9.94 [95%CI: −16.44 to −3.45], p = 0.003; estimated score (self-report): 97.57, β = −19.15 [95%CI: −26.82 to −11.48], p < 0.001). From this, it can be concluded that VIPN results in a significant reduction of HR-QoL in children under treatment for a malignancy, which means that VIPN has an important adverse effect on the well-being of pediatric oncology patients. This study underlines the importance of optimizing treatment with VCR, thereby aiming to reduce VIPN while maintaining treatment efficacy.

Chapter 5 describes the results of an intervention study investigating the effect of administration duration of VCR on VIPN development in children with cancer. In this study, one-hour infusions are compared to push-injections with respect to their effect on VIPN. This is a multicenter randomized controlled trial in which participants received all VCR administrations through push injections or one-hour infusions. VIPN was measured similar to the measurements described in **Chapter 4**. Moreover, data on comedication, such as azole antifungals, were collected. Overall, results show no effect of administration duration on total CTCAE score or ped-mTNS score. However, total CTCAE score is significantly lower in patients receiving one-hour infusions concurrently treated with azole antifungal therapy (β = 1.58; p = 0.04). Therefore, it is concluded that based on this study VCR administration through one-hour infusions do not result in less VIPN compared to VCR push injections in pediatric oncology patients overall. However, one-hour infusion leads to less severe VIPN compared to push-injections when azole antifungal therapy is administered concurrently with VCR. Therefore, children treated with VCR and requiring concurrent azole therapy likely benefit from VCR one-hour infusions instead of push injections, although larger trials are needed to confirm this association.

Chapter 6 describes the effects of administration duration (push injections 1-5 minutes versus one-hour infusions) on VCR pharmacokinetics (PK), and the association between PK and VIPN. PK was assessed at 1–5 occasions (1–8 samples in 24 h per occasion). PK samples were analyzed using high-performance liquid chromatography/tandem mass spectrometry. Population PK of VCR and its relationship with administration duration was determined using a linear two-compartment model with first-order elimination and data was analyzed using a non-linear mixed effect model. Furthermore, individual post-hoc parameters were estimated: area under the concentration time curve (AUC) and maximum concentration (C_{max}) in the plasma and in the

peripheral compartment. VIPN was assessed as described in **Chapter 4** and **Chapter 5**.The intercompartmental clearance (IC-Cl), volume of the peripheral compartment (V_2), and C_{max} are significantly higher in the push group. Furthermore, higher IC-Cl is significantly correlated with VIPN development. Therefore, it can be concluded that administration of VCR by push leads to increased IC-Cl, V_2, and C_{max} compared to one-hour infusions. However, AUC is similar between push injections and one-hour infusions. In conclusion, administration of VCR by one-hour infusions leads to similar or higher exposure of VCR without increasing VIPN.

Some children are more susceptible to VIPN, depending on genetic factors and PK. Some genetic variants leading to VIPN have already been identified, but no genetic factors leading to VCR PK differences are currently known. **Chapter 7** describes the results of a gene-targeted approach investigating associations between genetic factors and VCR PK and VIPN. In this study, genetic associations were identified and validated between VCR and VIPN and VCR and PK in children with cancer. VIPN was, again, measured as in **Chapter 4**, **Chapter 5** and **Chapter 6** using two tools: CTCAE and the ped-mTNS. The analyzed PK samples of **Chapter 6** were used for investigating its association with genetic factors. Targeted next-generation sequencing was performed and single nucleotide polymorphisms (SNPs) were studied in 94 pre-identified genes, which were selected based on present literature or protein function. In total n=85 patients were included in the study. Nine single-nucleotide polymorphisms (SNPs) in seven genes (NDRG1, GARS, FIG4, FGD4, SEPTIN9, CEP72 and ETAA1) are associated with VIPN. Furthermore, three SNPs in three genes (MTNR1B, RAB7A and SNU13) were associated with PK of VCR. In conclusion, PK of VCR and VIPN are influenced by SNPs; upfront identification of those that lead to an altered susceptibility to VIPN or VCR exposure could help individualize VCR treatment.

Chapter 8 describes a study protocol aimed towards an alternative VCR dosing regimen for children at low risk of developing VIPN. This study, which is scheduled to open for accrual in late 2022, will be conducted in Moi Teaching and Referral Hospital in Eldoret, Kenya. It is known that in clinical practice, VCR is often sub-optimally dosed in black African children compared to Caucasian children due to genetic differences in the metabolism of VCR. This study aims to optimize the dosing regimen of VCR using therapeutic drug monitoring while carefully monitoring toxicity. This will be a prospective cohort study. All children aged 2-14 years diagnosed with cancer and scheduled to receive a minimum of four administrations of VCR can be included. After the administration of VCR, blood samples will be taken. These will be shipped to and analyzed in the Netherlands to determine the VCR concentration in each sample. Based on this, a dose advise will be given for subsequent VCR administrations. This

cycle (VCR administration – sampling – analysis – adapted VCR dose) will be repeated maximum three times in total, when children receive sufficient VCR administrations according to their treatment protocol. Toxicity will be monitored by determination of the bilirubin, by CTCAE and by ped-mTNS assessment. This study aims to include 100 patients. The required dose to achieve a therapeutic VCR level, will be compared to a historic cohort of mainly Caucasian patients. The results could lead to an alteration in the dosing regimen of VCR in black (African) pediatric oncology patients, thereby improving overall therapeutic efficacy and treatment outcome of all black (African) pediatric oncology patients.

Chapter 9 puts all results of this thesis into perspective and addresses the implications of the various study results. The lack of consistency in measurement tools to assess VIPN is discussed, which seriously hampers the comparability between studies. It is advocated that a patient-reported outcome measurement (PROM) for VIPN assessment in children should be developed and thoroughly studied for its validity and reliability. This could possibly lead to the identification of a golden standard for VIPN assessment. Furthermore, the consequences of VIPN are discussed, mainly the effect on HR-QoL, both during and after treatment. The notion that VIPN leads to reduced HR-QoL during treatment should contribute to more efforts towards optimization of VCR treatment in children. One of the options to optimize VCR treatment is determination of the optimal VCR administration duration. Although this effect is not unequivocally demonstrated, in case concomitant treatment with azole antifungals is needed, one-hour infusions of VCR should be considered. Furthermore, two important factors are discussed that could lead towards a more individualized treatment regimen of VCR: PK and genetic associations. More generally, it is also discussed why it is important to focus on optimization of current anticancer treatment especially in children and not solely on development of new drugs. Moreover, the strengths and limitations of the presented studies in this thesis are discussed,. Currently undertaken study endeavors are also discussed, such as the study mentioned in **Chapter 8**. Finally, it is advocated that there is a need for a more individualized dosing regimen for VCR in children, which aims to optimize the therapeutic efficacy and minimize the toxicity of VCR.

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Nederlandse samenvatting

In dit proefschrift zijn verschillende aspecten van vincristine (VCR)-geïnduceerde perifere neuropathie (VIPN) onderzocht. VCR is een veelgebruikt chemotherapeuticum binnen de kinderoncologie. De belangrijkste bijwerking is VIPN. Dit is een ernstige dosis-beperkende bijwerking die zorgt voor sensorische symptomen (zoals pijn, dofheid en tintelingen) en/of motorische symptomen (zoals een klapvoet of moeilijkheden met lopen). Bovendien kan VIPN autonome symptomen veroorzaken, zoals obstipatie. Omdat VCR frequent gebruikt wordt in meerdere kinderoncologische behandelprotocollen, hebben veel kinderen te maken met deze bijwerkingen. Met de studies uit dit proefschrift willen we nieuw licht schijnen op welke kinderen een verhoogd risico lopen op het ontwikkelen van VIPN, de manier waarop we VIPN meten bij kinderen, de relatie tussen farmacokinetiek (PK) van VCR en VIPN en de relatie tussen genetische factoren en VIPN. Tevens beschrijft dit proefschrift de uitkomsten van een interventiestudie welke als doel had om VIPN in kinderen te verminderen en een studie waarin de gevolgen van VIPN op de kwaliteit van leven (HR-QoL) gedurende de behandeling is onderzocht. Tenslotte wordt de opzet van een nieuwe interventiestudie beschreven waarin het optimaliseren van de VCR behandeling in een specifieke populatie wordt onderzocht.

Kinderkanker is een zeldzame ziekte. Bij kinderen komen andere types kanker voor dan bij volwassenen. Bij kinderen is acute lymfatische leukemie de meest voorkomende vorm van kanker, terwijl bij volwassenen met name solide tumoren voorkomen en dit betreffen vaak carcinomen. Deze carcinomen zijn echter zeldzaam bij kinderen. De overleving van kinderkanker is de laatste decennia sterk verbeterd, waardoor er een verschuiving heeft plaatsgevonden in het onderzoeksveld, waarbij er niet langer alleen op de overleving van kinderkanker wordt gefocust, maar ook op de kwaliteit van overleving. VCR is één van de oudste types van chemotherapie. Het is reeds in de jaren zestig ontdekt en het is afkomstig uit de *Catharanthus roseus* plant. Het werkingsmechanisme is gebaseerd op het remmen van mitose, of celdeling, in verschillende celtypen. VIPN kent een complexe pathofysiologie: VCR zorgt voor veranderingen in het immuunsysteem die uiteindelijk leiden tot neuro-inflammatie. Het verandert ook actiepotentialen en de gevoeligheid voor prikkels in de zenuwen. Tenslotte zorgt VCR voor een afname van het aantal sensorische vezels in de zenuwen en een verandering in het metabolisme van de zenuwen. Er is geen specifieke behandeling voor VIPN, er zijn alleen ondersteunende behandelingen van de symptomen van VIPN. Het werkingsmechanisme van VCR, de pathofysiologie van VIPN en de factoren die bijdragen aan de ontwikkeling van VIPN zijn in meer detail beschreven in **Hoofdstuk 1**.

In **Hoofdstuk 2** wordt in een systematische review een overzicht gegeven van de beschikbare literatuur over: de verschillende manieren om VIPN te meten in kinderen

en de factoren die van invloed zijn op het ontwikkelen van VIPN. Hierbij is er gekeken naar verschillende patiëntkarakteristieken (zoals leeftijd en geslacht), maar ook naar de gebruikte dosering van VCR, de toedieningsduur, PK en genetische factoren. Er zijn 28 studies geïncludeerd. Allereerst zijn de verschillende meetmethodes van VIPN beschreven, met de bijbehorende psychometrische eigenschappen. Het gebruik van een scala aan meetmethodes voor VIPN zorgt ervoor dat het lastig is om de resultaten van verschillende studies met elkaar te vergelijken. Verder toonden de resultaten van deze review aan dat Kaukasische afkomst, een hogere VCR dosering, hogere leeftijd en een tragere klaring van VCR leiden tot een verhoogd risico op VIPN, al zijn de resultaten van de laatste twee factoren niet eenduidig. Tevens zijn er genetische factoren geïdentificeerd die VIPN beïnvloeden. In dit Hoofdstuk is een schematisch overzicht gepresenteerd van alle factoren die van invloed zijn op VIPN. Deze factoren, evenals hun onderliggende relaties, zijn allen van belang voor het ontstaan van (ernstige) VIPN bij kinderen.

In **Hoofdstuk 3** worden de resultaten beschreven van de vertaling van een Nederlands meetinstrument van VIPN, namelijk de pediatric-modified Total Neuropathy Score (ped-mTNS). De ped-mTNS is onderzocht op de constructvaliditeit en betrouwbaarheid in een Nederlandse populatie van kinderen met kanker tussen de 5-18 jaar oud. De constructvaliditeit (primaire uitkomstmaat) van de ped-mTNS is bepaald aan de hand van de verwachte correlatie tussen scores van 2 meetinstrumenten: de ped-mTNS (scoremogelijkheden: 0-32) en de Common Terminology Criteria for Adverse Events (CTCAE) (scoremogelijkheden: 0-18). De metingen zijn gedaan bij zowel patiënten als bij gezonde controles. Inter-rater- en intra-rater betrouwbaarheid en meetfout (secundaire uitkomstmaten) zijn bepaald in een subgroep van patiënten. Uit de resultaten blijkt dat in de groep van 112 kinderen (56 patiënten en 56 controles van dezelfde leeftijd en geslacht) de correlatie tussen de CTCAE en de ped-mTNS scores was zoals van te voren verwacht (namelijk gemiddeld ($r = 0.60$)). Tevens hadden de patiënten, zoals ook van tevoren verwacht, een significant hogere ped-mTNS score en ervaarden zij vaker symptomen van VIPN in vergelijking met de controles ($p < 0.001$). De betrouwbaarheid is gemeten binnen de intra-rater subgroep ($n = 10$). De intra-class correlation coefficient ($ICC_{agreement}$) was 0.64, de standard error of measurement ($SEM_{agreement}$) was 2.92 en de smallest detectable change ($SDC_{agreement}$) was 8.1. Binnen de inter-rater groep ($n = 10$) bleek de $ICC_{agreement} = 0.63$, $SEM_{agreement} = 3.7$ en $SDC_{agreement} = 10.26$. Deze resultaten suggereren een onvoldoende betrouwbaarheid van de Nederlandse versie van de ped-mTNS. Echter, de constructvaliditeit van dit meetinstrument blijkt goed te zijn. Concluderend is het nodig is om het meten van VIPN bij kinderen te standaardiseren. Toekomstig onderzoek zou gericht moeten zijn op het verbeteren van de psychometrische eigenschappen van de ped-mTNS.

In **Hoofdstuk 4** is het effect van VIPN op kwaliteit van leven (HR-QoL) gedurende de behandeling van kinderkanker beschreven. VIPN is gemeten voor start behandeling en 1-5 keer gedurende de behandeling middels de CTCAE en de ped-mTNS. HR-QoL is gemeten met zelf- en proxy-assessment van de Cancer en de Generic module van de Pediatric Cancer Quality of Life Inventory™ (PedsQL). In totaal zijn er 86 patiënten in deze studie geïncludeerd. HR-QoL van kinderen met VIPN (n = 67, 76%) was significant lager in vergelijking met kinderen zonder VIPN: benaderde Totale score van de PedsQL Generic (proxy) 84.57; β = −8.96 (95% betrouwbaarheidsinterval (CI) −14.48 tot −3.43); p = 0.002, benaderde PedsQL Generic Total score (zelf): 85.16, β = −8.38 (95% CI: −13.76 tot −3.00); p = 0.003. Vergelijkbare resultaten werden gevonden in het Pain domein van de PedsQL Cancer (benaderde score (proxy): 85.28, β = −9.94 (95%CI: −16.44 tot −3.45), p = 0.003; benaderde score (zelf) 97.57, β = −19.15 (95%CI: −26.82 tot −11.48), p < 0.001. Hieruit kan geconcludeerd worden dat VIPN leidt tot een significante afname van HR-QoL in kinderen die behandeld worden voor een vorm van kinderkanker, wat betekent dat VIPN belangrijk is voor het welzijn van kinderen met kanker. Deze studie onderstreept het belang van het optimaliseren van de behandeling met VCR, waarbij het doel moet zijn om VIPN te verminderen en de effectiviteit van VCR gelijk te houden.

In **Hoofdstuk 5** wordt een interventiestudie gepresenteerd naar het effect van toedieningsduur van VCR op de ontwikkeling van VIPN bij kinderen met kanker. In deze studie zijn één-uurs infusies vergeleken met push-injecties op de ontwikkeling van VIPN. Het betreft een gerandomiseerde multicenter studie waarin patiënten al hun VCR toedieningen of als push-injectie of als één-uurs infusie hebben ontvangen. Hierbij is voor de meting van VIPN gebruik gemaakt van de dezelfde meetinstrumenten als die worden beschreven in **Hoofdstuk 5**. Ook is er data verzameld over comedicatie, zoals azolen antischimmel medicatie. Resultaten toonden geen effect van toedieningsduur op de totale CTCAE of ped-mTNS score, maar totale CTCAE score was wel significant lager in patiënten die VCR als één uurs infusie kregen en tevens azolen antischimmel gebruikten (β = 1.58; p = 0.04). Daarom is vanuit deze studie geconcludeerd dat één-uurs infusies van VCR niet leiden tot minder VIPN in vergelijking met VCR push injecties in kinderen met kanker. Indien echter tevens azolen antischimmelmedicatie gebruikt worden, zullen één-uurs infusies van VCR leiden tot minder VIPN in vergelijking met push injecties van VCR. Daarom zouden één-uurs infusies in plaats van push-injecties van VCR voordelig kunnen zijn als een kind tevens azolen antischimmel medicatie nodig heeft. Er zijn echter grotere studies nodig om deze relatie te bevestigen.

In **Hoofdstuk 6** wordt ook het effect van toedieningsduur (push-injecties versus één-uurs infusies) van VCR bestudeerd, maar dan op de PK van VCR. Ook wordt de associa-

tie tussen PK en VIPN bestudeerd. PK is gemeten op 1 tot 5 meetmomenten (1 tot 8 samples gedurende 24 uur per meetmoment). Samples zijn geanalyseerd met behulp van high-performance liquid chromatography/tandem mass spectrometry. Populatie PK van VCR en de relatie met toedieningsduur is bepaald met behulp van een lineair twee compartiment model met first-order eliminatie en geanalyseerd volgens de non-lineair mixed effect methode. Ook zijn er individuele post-hoc parameters berekend: area under the concentration time curve (AUC) en maximale VCR concentratie (C_{max}) in het plasma en het perifere compartiment. VIPN is gemeten zoals beschreven in **Hoofdstuk 4** en **Hoofdstuk 5**. In totaal zijn er 70 PK meetmomenten in 35 kinderen geanalyseerd. Resultaten toonden aan dat de intercompartmental clearance (IC-Cl), volume of the peripheral compartment (V_2), en C_{max} significant hoger waren in de push groep. Tevens was een hogere IC-Cl significant gecorreleerd met VIPN. Oftewel: push toedieningen leiden tot toename van IC-Cl, V_2 en C_{max} in vergelijking met één-uurs infusies, terwijl de AUC hetzelfde is tussen de beide groepen. Concluderend kan gesteld worden dat toediening van VCR in één uur tot eenzelfde of hogere blootstelling van VCR leidt zonder toename van VIPN.

Hoofdstuk 7 beschrijft de associatie tussen genetische factoren en de PK van VCR en VIPN. Uit de literatuur blijkt dat sommige kinderen gevoeliger zijn voor de ontwikkeling van VIPN. Dit komt met name door verschillen in genetische factoren en PK van VCR. Sommige genetische varianten die tot VIPN kunnen leiden zijn al geïdentificeerd maar er zijn nog geen genetische factoren geïdentificeerd die kunnen leiden tot PK-verschillen van VCR. In deze studie zijn genetische associaties geïdentificeerd en gevalideerd tussen VCR en VIPN en VCR en PK in kinderen met kanker. VIPN is gemeten zoals beschreven in **Hoofdstuk 4**, **Hoofdstuk 5** en **Hoofdstuk 6.** De geanalyseerde PK samples uit **Hoofdstuk 6** zijn gebruikt om de associatie met genetische factoren te bestuderen. Voor de genetische factoren is gebruik gemaakt van gerichte next-generation sequencing en zijn SNPs bestudeerd in 48 vooraf vastgestelde genen, welke zijn geselecteerd op bekende associaties in de huidige literatuur en/of de functie van het eiwit waarvoor een gen codeert. In totaal zijn 85 kinderen geïncludeerd in de studie. Uit de resultaten bleek dat er negen SNPs in 7 genen (NDRG1, GARS, FIG4, FGD4, SEPTIN9, CEP72 en ETAA1) geassocieerd waren met VIPN. Verder bleken er 3 SNPs in 3 genen (MTNR1B, RAB7A en SNU13) geassocieerd met PK van VCR. Uit de resultaten kan geconcludeerd worden dat de PK van VCR en VIPN beïnvloed worden door verschillende SNPs. Het identificeren van patiënten die voorafgaand aan de behandeling een verhoogd risico lopen op het ontwikkelen van VIPN of bijvoorbeeld een lagere blootstelling aan VCR kan er in de toekomst hopelijk voor zorgen dat de behandeling van VCR aangepast kan worden en zo leiden tot een betere behandeling met VCR.

In **Hoofdstuk 8** wordt het protocol van een studie gepresenteerd dat als belangrijkste doel heeft om de dosering strategie van VCR aan te passen in een groep kinderen met een verlaagd risico op VIPN. Deze studie zal worden uitgevoerd in het Moi Teaching and Referral Hospital in Eldoret, Kenia. In zwarte (Afrikaanse) kinderen is VCR vaak suboptimaal gedoseerd doordat dezelfde dosering wordt gebruikt als in witte Kaukasische kinderen, maar door genetische verschillen in het metabolisme van VCR hebben zwarte (Afrikaanse) kinderen vaak een lagere blootstelling aan VCR. In deze studie wordt de dosering van VCR geoptimaliseerd met behulp van therapeutic drug monitoring terwijl de toxiciteit zorgvuldig wordt gemonitord. Het is een prospectieve cohort studie. Alle kinderen tussen de 2-14 jaar die gediagnosticeerd worden met kanker en die volgens het behandelprotocol van hun ziekte een bepaald minimum aantal keren VCR toegediend krijgen, kunnen worden geïncludeerd. Na de toedieningen van VCR zullen bloedsamples worden afgenomen, welke naar Nederland worden verscheept en hier worden geanalyseerd om de spiegel van VCR in elk sample te bepalen. Gebaseerd op deze metingen kan vervolgens een dosisadvies worden gegeven voor de daaropvolgende VCR toedieningen. Deze cyclus (VCR toediening – samplen – analyseren – aanpassen VCR dosering) zal in totaal maximaal drie keer worden herhaald, indien er volgens het behandelprotocol voldoende VCR toedieningen zijn. Toxiciteit zal worden gemonitord met behulp van het bilirubinegehalte in het bloed, de CTCAE en de ped-mTNS. Voor deze studie zullen 100 kinderen worden geïncludeerd. De benodigde dosering om een therapeutische VCR concentratie te bereiken zal worden vergeleken met een historisch cohort van voornamelijk witte Kaukasische kinderen. De resultaten van deze studie kunnen leiden tot een aanpassing in de dosering van VCR in alle zwarte (Afrikaanse) kinderen met kanker, waarbij de effectiviteit en de uitkomst van de behandeling van kinderkanker in deze groep kan worden verbeterd.

In **Hoofdstuk 9** worden alle resultaten van dit proefschrift in perspectief geplaatst en worden de implicaties van de bevindingen besproken. Het gebrek aan consistentie om VIPN te meten wordt bediscussieerd, wat serieuze gevolgen heeft voor het vergelijken van studieresultaten. Er wordt beargumenteerd dat er een patient reported outcome measurement (PROM) voor VIPN-metingen in kinderen moet worden ontwikkeld, waarbij de validiteit en betrouwbaarheid ook zal moeten worden onderzocht. Dit alles om te komen tot een gouden standaard voor het meten van VIPN in kinderen. Ook de gevolgen van VIPN worden besproken, met name het effect op HR-QoL, zowel gedurende als na de behandeling. De uitkomst dat VIPN leidt tot een verminderde HR-QoL, geeft aan dat het van groot belang is om de VCR-behandeling van kinderen verder te optimaliseren. Eén van de opties om de behandeling te optimaliseren is het bepalen van de meest optimale toedieningsduur van VCR. Alhoewel dit effect niet onomstotelijk is aangetoond, moeten één uurs infusies worden overwogen indien

er naast VCR tevens azolen antischimmelmedicatie moet worden gebruikt. Tevens worden twee belangrijke determinanten besproken die kunnen leiden tot een meer geïndividualiseerde behandeling van VCR: PK en genetische factoren. In meer algemene zin wordt er tenslotte bediscussieerd waarom het belangrijk is om te focussen op het optimaliseren van de huidige oncologische behandeling, in het bijzonder bij kinderen, en niet alleen op het ontwikkelen van nieuwe medicatie. Ook de sterke en zwakke punten van de studies in dit proefschrift worden besproken. Tenslotte wordt de huidige stand van zaken besproken met betrekking tot het optimaliseren van de behandeling van VCR, waarbij de toekomstige studie zoals beschreven in **Hoofdstuk 8** ook aan bod komt. Als conclusie wordt er beargumenteerd dat er een noodzaak is voor een meer geïndividualiseerde behandeling van VCR in kinderen, waarbij de effectiviteit wordt geoptimaliseerd en de toxiciteit wordt geminimaliseerd.

ABBREVIATIONS

95% CI	95% Confidence Interval
ABC	ATP Binding Cassette
ALL	Acute Lymphoblastic Leukemia
allo-SCT	allogeneic Stem-Cell Transplant
ATP	Adenosine Triphosphate
AUC	Area Under the Concentration time curve
BFM	Berlin-Frankfurt-Munster
BOT-2	Bruininks-Osteretsky Test of motor proficiency version 2
BSA	Body Surface Area
Ca	Calcium
CCG	Children's Cancer Group
CEP72	Centrosomal Protein 72
Cl	Clearance
CMAP	Compound Muscle Action Potential
C_{max}	Maximum Concentration
CMT	Charcot-Marie Tooth
CNS	Central Nervous System
COG	Children's Oncology Group
CTCAE	Common Terminology Criteria for Adverse Events
CV	Coefficient of Variation
CYP	Cytochrome P450
DALY	Disability-Adjusted Life Years
DCOG	Dutch Childhood Oncology Group
DFCI	Dana-Farber Cancer Institute
DTR	Deep Tendon Reflexes
EBE	Empirical Bayesian Estimates
eQTL	expression Quantitative Trait Loci
ESI	Electrospray Ionization
ETAA1	Ewing's Tumor-Associated Antigen 1
FDR	False Discovery Rate
FGD4	FYVE, RhoGEF And PH Domain Containing 4
FIG4	FIG4 Phosphoinositide 5-Phosphatase
FLACC	Face, Legs, Activity, Cry, Consolability
GARS	Glycyl tRNA Synthetase
GEE	Generalized Estimating Equations
GWAS	Genome Wide Association Study
HLH	Hemophagocytic LymphoHistiocytosis
HPLC	High-Performance Liquid Chromatography
HR	High Risk

HR-QoL	Health-Related Quality of Life
ICC	Intra-Class Correlation coefficient
IC-Cl	Intercompartmental Clearance
IFI	Invasive Fungal Infection
IQR	InterQuartile Range
IR	Intermediate Risk
IRB	Institutional Review Board
K	Potassium
LCMS	Liquid Chromatography-tandem Mass Spectrometry
LGG	Low-Grade Glioma
MAF	Minor Allele Frequency
MIC	Minimal Important Change
miRNA	microRNA
MS	tandem Mass Spectrometry
MTNR1	Melatonin Receptor 1B
Na	Sodium
NCI CTC	National Cancer Institute Common Toxicity Criteria
NCS	Nerve Conduction Studies
NDRG1	N-Myc Downstream Regulated 1
NHP2L1/SNU13	Small Nuclear Ribonucleoprotein 13
NPS	Neuropathic Pain Scale
NWO	Dutch Organization for Scientific Research
OFV	Objective Function Value
OR	Odds Ratio
P-CIN	Pediatric Chemotherapy-Induced Neuropathy
Ped-mTNS	Pediatric-modified Total Neuropathy Score
PedsQL	Pediatric Cancer Quality of Life Inventory™
PIP	Pediatric Investigation Plan
PK	Pharmacokinetics
PNPS	Pediatric Neuropathic Pain Scale
PROM	Patient-Reported Outcome Measure
RCT	Randomized Clinical Trial
RMS	RhabdoMyoSarcoma
RSE	Relative Standard Error
SAEM	Stochastic Approximation Expectation-Maximization
SD	Standard Deviation
SD	Standard Deviation
SDC	Smallest Detectable Change
SEM	Standard Error of Measurement
SEPTIN9	Septin 9
SNAP	Sensory Nerve Action Potential

SNP	Single Nucleotide Polymorphism
SR	Standard Risk
SSEP	Somato Sensory Evoked Potentials
$t_{1/2}$	Half-life
Tenalea	Trans European Network for Clinical Trials Services
TNS	Total Neuropathy Score
TNS-PV	Total Neuropathy Score – Pediatric Version
TPMT	thiopurine S-methyltransferase
TRP	Transient Receptor Potential channel
V_1	Volume of distribution of the central
V_2	Volume of distribution of the peripheral compartment
VCR	Vincristine
Vd	Volume of Distribution
VDR	Vitamin D Receptor
VIPN	Vincristine Induced Peripheral Neuropathy
VPT	Vibration Perception Threshold
WHO	World Health Organization

LIST OF CO-AUTHORS

Abbink, F.C.H.	Emma Children's Hospital, Amsterdam UMC, Amsterdam Medical Center, Pediatric Oncology Amsterdam, The Netherlands
Bonten, E.	St. Jude Children's Research Hospital, Pharmaceutical Sciences, Memphis, Tennessee, USA
Chantrain, C.	Clinique du MontLégia, CHC, Pediatrics, Liège, Belgium
Cheng. C.	St. Jude Children's Research Hospital, Biostatistics, Memphis, Tennessee, USA
Evans, W.E.	St. Jude Children's Research Hospital, Pharmaceutical Sciences, Memphis, Tennessee, USA
Hartman, A.	Erasmus Medical Center Rotterdam/Sophia Children's Hospital, Pediatric Physiotherapy, Rotterdam, The Netherlands
Kaspers, G.J.L	Emma Children's Hospital, Amsterdam UMC, Vrije Universiteit Amsterdam, Pediatric oncology, Amsterdam, The Netherlands; Princess Máxima Center for Pediatric Oncology, Utrecht, The Netherlands
Ket, J.F.C.	Amsterdam UMC, Vrije Universiteit Amsterdam, Medical Library, Amsterdam, The Netherlands
Mokkink, L.B.	Amsterdam UMC, Vrije Universiteit Amsterdam, Epidemiology and Biostatistics and Amsterdam Public Health Research Institute, Amsterdam, The Netherlands
Panetta, J.C.	St. Jude Children's Research Hospital, Pharmaceutical Sciences, Memphis, Tennessee, USA
Pei, D.	St. Jude Children's Research Hospital, Biostatistics, Memphis, Tennessee, USA
Schouten, S.M.	Emma Children's Hospital, Amsterdam UMC, Vrije Universiteit Amsterdam, Pediatric oncology, Amsterdam, The Netherlands
Segers, H.	University Hospitals Leuven, Pediatric Hemato-Oncology, Leuven, Belgium;
Twisk, J.W.R.	Amsterdam UMC, Vrije Universiteit Amsterdam, Epidemiology and Biostatistics, Amsterdam, The Netherlands
Uittenboogaard, A.	Emma Children's Hospital, Amsterdam UMC, Vrije Universiteit Amsterdam, Pediatric oncology, Amsterdam, The Netherlands
van den Berg, M.H.	Emma Children's Hospital, Amsterdam UMC, Vrije Universiteit Amsterdam, Pediatric oncology, Amsterdam, The Netherlands
van den Bos, C.	Emma Children's Hospital, Amsterdam UMC, Amsterdam Medical Center, Pediatric Oncology Amsterdam, The Netherlands; Princess Máxima Center for Pediatric Oncology, Utrecht, The Netherlands
van den Heuvel-Eibrink, M.M.	Princess Máxima Center for Pediatric Oncology, Utrecht, The Netherlands
van der Sluis, I.M.	Princess Máxima Center for Pediatric Oncology, Utrecht, The Netherlands
van der Werff Ten Bosch, J.	Universitair Ziekenhuis Brussel, Pediatric Onco-Hematology, Brussel, Belgium
van Litsenburg, R.R.L.	Princess Máxima Center for Pediatric Oncology, Utrecht, The Netherlands
Wilhelm, A.J.	Amsterdam UMC, Vrije Universiteit Amsterdam, Clinical Pharmacology and Pharmacy Amsterdam, The Netherlands
Willems, L.	Ghent University Hospital, Paediatric Haematology-Oncology and Stem Cell Transplantation, Ghent, Belgium
Yang, W.	St. Jude Children's Research Hospital, Pharmaceutical Sciences, Memphis, Tennessee, USA

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PHD PORTFOLIO

Name PhD student: MSc, MD. M.E. van de Velde
PhD period: May 2014 – January 2022
Promotor: Prof. dr. G.J.L. Kaspers
Copromotor: Dr. M.H. van den Berg

PhD training	Year
Courses and workshops	
Basic Course Oncology, Dutch Association for Oncology (NVvO), Ellecom, the Netherlands	2014
Basic Course Legislation and Organization Clinical Research (BROK/GCP), VU University, Amsterdam, the Netherlands	2014
Advanced Pediatric Life Support course, 'Groepering voor Opvang van Kinderen In Nood', Brussel, Belgium	2017
Selected for TULIPS PhD curriculum	2017-2019
Re-registration Basis Course Legislation and Organization Clinical Research (BROK/GCP), VU University, Amsterdam, the Netherlands	2018
Longitudinal analyses, EpidM, VU University, Amsterdam, the Netherlands	2019
Newborn Life Support course, 'Stichting Spoedeisende Hulp bij Kinderen', Riel, the Netherlands	2021
Presentations	
The VINCA trial. 'Amsterdam Kinder Symposium'. Amsterdam, the Netherlands. Oral presentation	2015
Carboplatin dosing in children using estimated glomerular filtration rate: equation matters. European Society for Paediatric Nephrology Conference, Glasgow, Scotland. Poster presentation	2017
Population pharmacokinetics of vincristine administered through push-injections and one-hour infusions in pediatric oncology patients. International Society of Pediatric Oncology (SIOP) conference. Lyon, France. Poster presentation	2019
Vincristine-Induced Peripheral Neuropathy in Pediatric Oncology Patients Receiving vincristine Through Push Injections or One-Hour Infusions: Results of a Randomized Clinical Trial. American Society for Hematology Conference, Orlando, Florida, United States of America. Poster presentation.	2019
Vincristine-induced peripheral neuropathy is associated with reduced health-related quality of life in pediatric oncology patients. International Society of Pediatric Oncology (SIOP) conference. Online (planned: Ottawa, Canada). Poster presentation	2020
Attendee (inter)national conferences	
'Amsterdam Kinder Symposium' Amsterdam, the Netherlands	2014-2018
Dutch Childhood Oncology Group/Princess Máxima Center retreat, De Bilt, the Netherlands	2015
TULIPS Child Health Symposium, Noordwijk, the Netherlands	2016
Annual conference of the Dutch Childhood Oncology Group, Utrecht, the Netherlands	2015-2017
International Annual Workshop on Pediatric Oncology, Eldoret, Kenya	2017
The 50th European Society for Paediatric Nephrology Conference, Glasgow, Scotland	2017

KiKA-Princess Máxima Center Pediatric Oncology Research Meeting/Tom Voûte Award election, Utrecht, the Netherlands	2017
St. Jude Children's Research Hospital annual Biomedical Research Symposium, Memphis, Tennessee, United States of America	2018
60th Annual American Society for Hematology conference, San Diego, California, United States of America	2018
51st International Society of Pediatric Oncology (SIOP) Conference, Lyon, France	2019
61st Annual American Society for Hematology conference, Orlando, Florida, California, United States of America	2019
52nd International Society of Pediatric Oncology (SIOP) Conference, Online, (Planned: Ottawa, Canada)	2020
'Nederlandse Vereniging voor Kindergeneeskunde' Conference, Online, the Netherlands	2021
Tutoring and supervising	
Regular research internship (bio)medical student, 6x	2015-2019
Bachelor thesis 3x	2015-2016
Prolonged research internship, honors program medical student, 1x	2015
Tutoring of third year medical students (± 13 students) during weekly study groups	2016
Grants	
Fulbright scholarship	2017
Ter Meulen scholarship	2017
René Vogels scholarship	2017
Cancer Canter Amsterdam Travelgrant	2017
Other	
Development of a new Minor in Pediatric Oncology for bachelor students	2016-2017
Member of organizing committee 'Amsterdam Kinder Symposium'	2016-2018

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ABOUT THE AUTHOR

Mirjam Esther van de Velde was born on March 23rd 1987 in Haarlem, the Netherlands. With her parents and two big brothers she grew up in Aerdenhout. She graduated from secondary school R.K. Lyceum Sancta Maria (Gymnasium) in Haarlem. In 2014 she gained her Master's degree in Medicine at the Vrije Universiteit in Amsterdam. After graduating, she started as a PhD student under the supervision of prof. Gertjan L. Kaspers and dr. Marleen H. van den Berg coordinating the VINCA trial: a clinical trial studying the effect of administration duration of vincristine on the development of vincristine induced peripheral neuropathy in children. Her international ambitions became already apparent during her medical training and continued during her PhD. She did clinical rotations on Pediatrics and Gynaecology on Aruba and as a family physician in Paramaribo, Surinam. During her PhD she started a clinical trial on therapeutic drug monitoring of vincristine in Eldoret, Kenya and gained experience as a preclinical scientist at St. Jude Children's Research Hospital in Memphis, Tennessee. Also, during her PhD she followed the TULIPS PhD program (a highly competitive trainee program for researchers in pediatrics) and organized the Amsterdam Kindersymposium twice. In 2021 she started her training as a resident in pediatrics at the Noordwest Ziekenhuisgroep in Alkmaar as part of her training program at Amsterdam UMC. She lives together with her wife Linda van de Velde – Bosschers and her two sons Nathan and Jesse in Baambrugge.