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# Hand-arm vibration injury - Neurosensory, vascular manifestations, severity grading, and serum biomarkers

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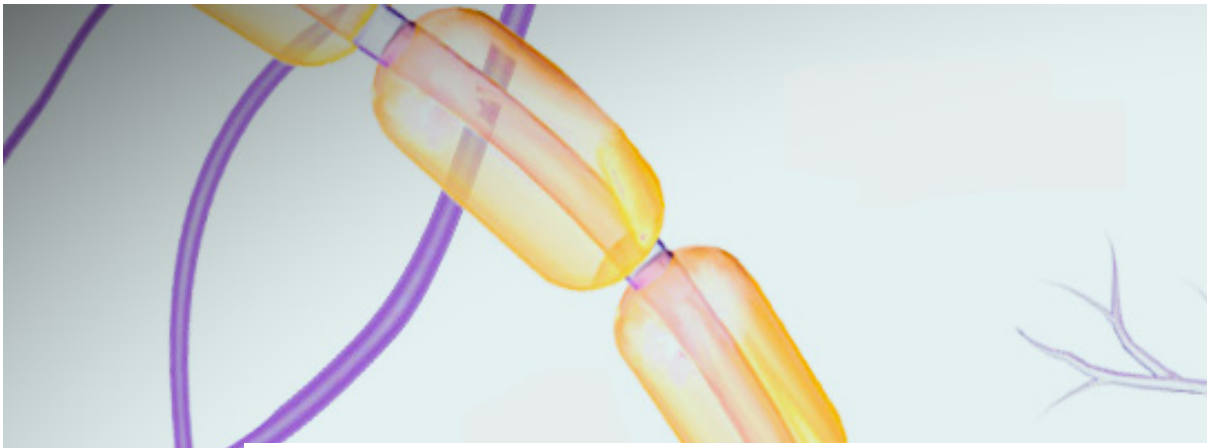
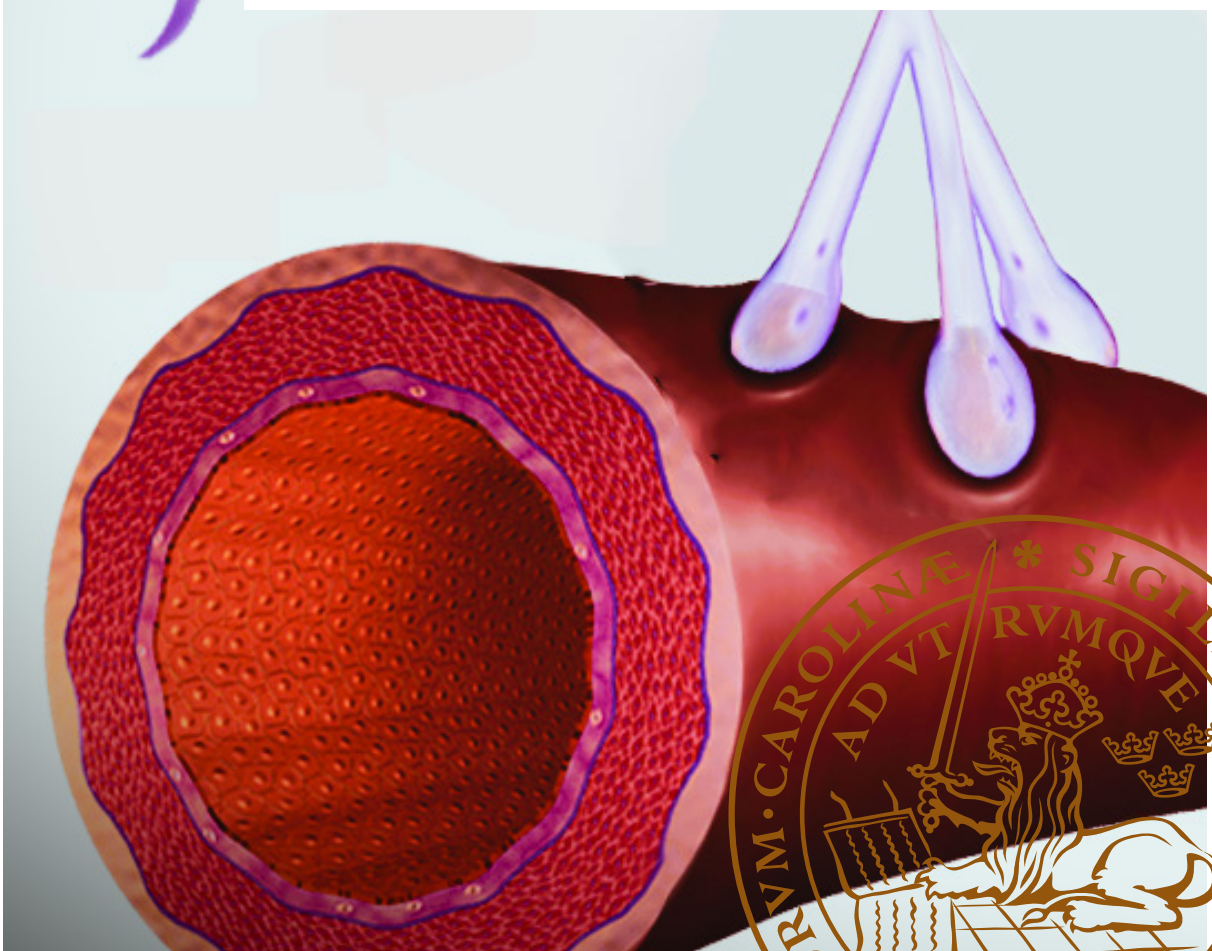


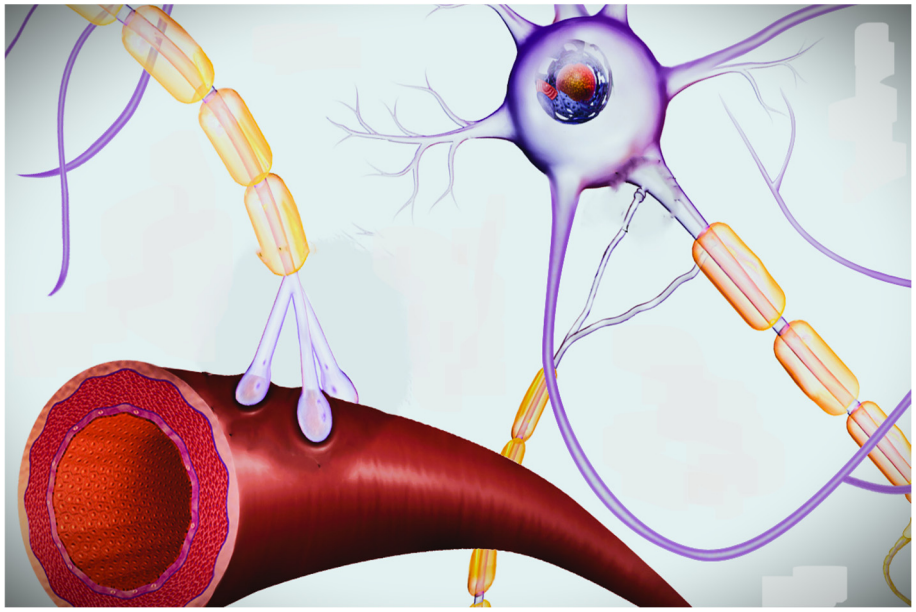
# Hand-arm vibration injury

## Neurosensory and vascular manifestations, severity grading, and serum biomarkers

EVA TEKAVEC
DIVISION OF OCCUPATIONAL AND ENVIRONMENTAL MEDICINE | LUND UNIVERSITY



# Hand-arm vibration injury



# Hand-arm vibration injury

## Neurosensory and vascular manifestations, severity grading, and serum biomarkers

Eva Tekavec

LUNDS UNIVERSITET

DOCTORAL DISSERTATION

Doctoral dissertation for the degree of Doctor of Philosophy (PhD) at the Faculty of Medicine at Lund University to be publicly defended on 29th of February at 13.00 in Sharience, The Spark, Medicon Village, Lund

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**Background** Despite EU directives stipulating regular helath surveillance and risk assessement, many workers exposed to hand-held vibrating tools get injured. Early detection is crucial for progosis and medical advice. However diagnosis and grading of injury are based on semiobjective methods. **Aims** *Paper I:* To describe the clinical picture of adverse health manifestations in the hands, among carpenters at their workplaces and among patients diagnosed with hand-arm vibration injury. *Paper II:* To compare two scales used for grading the neurosensory manifestations. *Paper III and IV*: To assess if serum-biomarkers could be used as an objective method in diagnostics and grading of injury. **Methods** *Paper I:* 193 carpenters and 72 painters answered a questionnaire and were examined. *Paper II:* Two neurosensory severity grading systems; the Stockholm workshop scale and the International concensus criteria were applied on 92 patients diagnosed with hand-arm vibration injury. *Paper III and IV*: Serum levels of biomarkers were assessed in 92 patients and 51 controls. **Results** *Paper I:* One third of carpenters (12% young carpenters) showed signs of injuries to their hands and compared to painters the odds ratio was threefold. *Paper II*: ICC resulted in lower grading scores.The clinical picture among both carpenters and patients dominated by neurosensory manifestations. Half of patients had Raynauds phenomenon (RP), 9% of carpenters. Cold intolerance was a common symptom, 86% among patients and 24% among carpenters. *Paper III and IV*: Biomarkers associated with inflammation, endothelial injury or dysfunction: intercellullar adhesion molecule 1 (ICAM-1), monocyte chemoactracctant protein 1 (MCP-1), and thrombomodulin (TM), and neuroprotection: heat shock protein 27 (HSP27) were elevated in vibration injured patients compared to controls. Comparing patients with RP and without RP; TM, and biomarkers for inflammation: von Willebrands factor (vWf), compensatory mechanisms; calcitonin gene related peptite (CGRP) and apoptosis; caspase-3 were elevated. TM, vWf, CGRP, HSP27 and caspase-3 were associated with the severity of RP. The associations were linear for TM, vWf, and Caspase, for HSP27 the association, was not linear. CGRP was associated with the severity of neurosensory manifestations. **Clinical significance** The results are important in the preventive work and risk communication with regulatory boards and for health care professionals, to be aware of early signs and symptoms of an occupational acquired disease in patients seeking for problems in their hands. Extended knowledge on pathophysiological mechanisms contributes to a more precise and timely diagnosis and to the development of specific treatments. Since young carpenters showed signs of injury this indicats that today's exposure is not safe.

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## Neurosensory and vascular manifestations, severity grading, and serum biomarkers

Eva Tekavec


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# Abstract

**Background** Despite EU directives stipulating regular helath surveillance and risk assessement, many workers exposed to hand-held vibrating tools get injured. Early detection is crucial for progosis and medical advice. However diagnosis and grading of injury are based on semiobjective methods. **Aims** *Paper I:* To describe the clinical picture of adverse health manifestations in the hands, among carpenters at their workplaces and among patients diagnosed with hand-arm vibration injury. *Paper II:* To compare two scales used for grading the neurosensory manifestations. *Paper III and IV*: To assess if serum-biomarkers could be used as an objective method in diagnostics and grading of injury. **Methods** *Paper I:* 193 carpenters and 72 painters answered a questionnaire and were examined. *Paper II:* Two neurosensory severity grading systems, the Stockholm workshop scale and the International concensus criteria were applied on 92 patients diagnosed with hand-arm vibration injury. *Paper III and IV*: serum levels of biomarkers were assessed in 92 patients and 51 controls. **Results** *Paper I:* One third of carpenters (12% young carpenters) showed signs of injuries to their hands. Compared to painters the odds ratio was threefold. *Paper II*: ICC resulted in lower grading scores.The clinical picture among both carpenters and patients dominated by neurosensory manifestations. Half of the patients had Raynaud´s phenomenon (RP) and 9% of the carpenters. Cold intolerance was a common symptom, 86% among the patients and 24% among the carpenters. *Paper III- IV*: Biomarkers associated with inflammation, endothelial injury or dysfunction; intercellullar adhesion molecule 1 (ICAM-1), monocyte chemoattractant protein 1 (MCP-1), thrombomodulin (TM), and neuroprotection; heat shock protein 27 (HSP27), were elevated in vibration injured patients compared to controls. Comparing patients with RP and without RP; TM, and biomarkers linked to inflammation; von Willebrand factor (vWf), compensatory mechanisms; calcitonin gene related peptide (CGRP) and apoptosis; caspase-3 were elevated. TM, vWf, CGRP, HSP27 and caspase-3 were associated with the severity of RP. There were linear associations for TM, vWf, and caspase-3, while for HSP27 there was an association, but not linear. CGRP was associated with the severity of neurosensory manifestations. **Clinical significance** The results are important in the preventive work and risk communication with regulatory boards and for health care professionals, to be aware of early signs and symptoms of an occupational acquired disease in patients seeking for problems in their hands. Extended kowledge about pathophysiological mechanisms contributes to a more precice and timely diagnosis and to the development of specific treatments. Since young carpenters showed signs of injury this indicats that today's exposure is not safe.

# Populärvetenskaplig sammanfattning

Trots regelverk för att skydda arbetstagare, omfattande bl. a gränsvärden för exponering och medicinska kontroller, skadas många i sitt arbete p g a exponering för vibrationer från handhållna verktyg. Vibrationsskada är den vanligaste godkända arbetssjukdomen hos män (38 %) och då är inte karpaltunnelsyndrom som kan ingå i diagnosen medräknat (24 %) (AFA försäkring, arbetssjukdom 2017-21) [1]. Att upptäcka tidig skada är avgörande för medicinsk uppföljning, prognos och socioekonomiska konsekvenser. Diagnos och gradering av allvarlighetsgraden baseras på upplevda symtom hos patienten i kombination med semiobjektiva undersökningsfynd. En objektiv mätmetod som till exempel förändrar halt av biomarkörer skulle kunna vara ett sätt att stärka diagnostik och kunna upptäcka skada tidigt redan innan den är mätbar vid nuvarande diagnostiska känseltest. Att studera ändrad halt av biomarkörer associerade med kärl respektive nervskada skulle även leda till ökad kunskap om pathofysiologiska mekanismer och bidra till ökad kunskap om prognos och individuell känslighet. I dagsläget saknas det effektiv behandling vid etablerad skada.

Vi undersökte snickare ute på deras arbetsplatser och fann att en tredjedel uppvisade tecken på skada i händerna. Jämfört med målare motsvarande det en trefaldig oddskvot. Eftersom snickare har avsevärt högre vibrationsexponering än målare, kan resultaten bero på vibrationsexponeringen. Då 12 % av unga snickare var skadade, indikerar detta att dagens regelverk för att skydda arbetstagarna är otillräckligt.

Patienter med hand-arm vibrationsskada graderades enligt två olika graderingssystem för nervskada; Stockholm workshopskalan (SWS SN) och International consensus criteria (ICC N). Det visade sig att 60 % av patienterna flyttade ner ett steg i allvarlighetsgrad enligt ICC Resultatet kan påverka den medicinska bedömningen och i vissa länder även påverka rätten till arbetsskade-ersättning

Den kliniska bilden av hand-arm vibrationsskada, både ute i fält bland snickare och bland diagnostiserade patienter dominerades av nervskada. Detta överensstämmer med gällande dos-responssamband där nervskadan föregår den vaskulära skadan (Raynaud´s fenomen). Prevalensen av Raynaud´s fenomen var 49 % bland patienter och 9 % bland snickare och endast 2 % bland snickare yngre än 30 år. Köldintolerans var ett mycket vanligt symtom (86 % bland patienterna och 24 % bland snickarna.

I blodprovsstudien uppvisade patienter med vibrationsskada högre serumhalter av biomarkörer som kan associeras med inflammation, skada eller påverkan på inre kärlväggen; intercellullar adhesion molecule 1 (ICAM-1), monocyte chemoattractant protein 1 (MCP-1), trombomodulin (TM). Heat shock proteiner är markörer som förknippas med skyddsmekanismer vid stress, heat shock protein 27 (HSP27) har setts i ökad halt vid nervskada. Vi fann att patienter med vibrationsskada hade högre halt av HSP27 jämfört med kontroller. Patienter med

Raynaud´s fenomen hade förutom högre halt av TM, även högre serumhalter av biomarkörer som kan associeras med inflammation och påverkan på kärlväggen (vWf), kompensatoriska mekanismer och apoptos (CGRP och caspase-3), jämfört med patienter utan Raynaud´s fenomen. Halten av TM, vWf och HSP27 och caspase-3 ökade med allvarlighetsgraden av Raynaud´s fenomen och CGRP ökade med allvarlighetsgraden av nervskadan.
Resultaten har gett ökade kunskaper om pathofysiologiska mekanismer som om de håller vid upprepade studier kan leda till utveckling av bättre diagnostik och behandling av patienter med hand-arm vibrationsskada.

# List of Publications

This thesis is based on the following papers, referred to in the text by their Roman numerals. The original articles have been reprinted with permission of the publisher or are open access.

**Paper I** Tekavec E, Löfqvist L, Larsson A, Fisk K, Riddar J, Nilsson T, Nordander C. Adverse health manifestations in the hands of vibration exposed carpenters - a cross sectional study. J Occup Med Toxicol. 2021 Apr 29;16(1):16.

**Paper II** Tekavec E, Nilsson T, Riddar J, Axmon A, Nordander C. Concordance between the Stockholm Workshop Scale and the International Consensus Criteria for grading the severity of neurosensory manifestations in hand-arm vibration syndrome in a Swedish clinical setting. Occupational and Environmental Medicine 2023;80:418-424.

**Paper III** Tekavec E, Nilsson T, Dahlin L B, Huynh E, Axmon A, Nordander C, Riddar J, Kåredal M. Serum biomarkers in patients with hand-arm vibration injury and in controls. Accepted for publication in Scientific Reports.

**Paper IV** Tekavec E, Nilsson T, Dahlin L B, Huynh E, Nordander C, Riddar J, Kåredal M. Serum levels of biomarkers in relation to severity staging of Raynaud's phenomenon, neurosensory manifestations, and vibration exposure, in patients with hand-arm vibration injury, Manuscript.

# Abbreviations

| | |
|---|---|
| HAVS | Hand-arm vibration syndrome |
| RP | Raynaud ’s phenomenon |
| CTS | Carpal tunnel syndrome |
| SWS | Stockholm workshop scale |
| ICC | International consensus criteria |
| OEM | Occupational and Environmental Medicine |
| QST | Quantitative sensory test |
| VPT | Vibration perception thresholds |
| ENeG | Electroneurography |
| TM | Thrombomodulin |
| SC | Schwann cell |
| vWf | von Willebrand factor |
| Et-1 | Endothelin-1 |
| CGRP | Calcitonin gene related peptide |
| HSP27 | Heat shock protein |
| GFAP | Glial fibrillary acidic protein |
| MBP | Myelin basic protein |
| TRPA-1 | Transient receptor potential channel ankyrin 1 |
| ICAM-1 | Intercellular adhesion molecule 1 |
| MCP-1 | Monocyte chemoattractant protein 1 |
| VEGF | Vascular endothelial growth factor |
| b-FGF | Basic fibroblast growth factor |
| HGF | Hepatocyte growth factor |
| MMP-1 | Metalloproteinase-1 |
| MMP-12 | Metalloproteinase-12 |
| β-NGF | Beta-neurotrophic factor |
| NT-3 | Neurotrophic factor-3 |
| BDNF | Brain-derived neurotrophic factor |

# Introduction

Workers exposed to handheld vibrating tools are under risk of contracting hand-arm vibration injury. In Europe an average of 5 million workers are exposed [2]. In Sweden about 400 000 workers, corresponding to 13% of all men and 3% of all women in the Swedish workforce are daily exposed to handheld vibrating tools for at least 25% of their workday. The construction sector is a highly exposed (70%) and male dominated work force [3]. Companies´ endeavour for cost-effective timelines enforce workers to handle the same work tool for extended time without breaks or job rotation.

Once symptoms from hands become manifest there is little improvement even if exposure completely stops [4]. Besides great individual harm, injured workers are enforced to quit their jobs, entailing socioeconomic consequences by workers compensation claims. Hand-arm vibration injury is the most approved occupational disease among Swedish men (38 %). On second place is carpal tunnel syndrome (24 %), a conditions also related to vibration exposure [1].

*Risk assessment and health surveillance*

In 2005 the European Union Vibration Directive 2002/44/EC was implemented in Sweden [5]. The Swedish surveillance system aiming at detecting early injury in workers exposed to hand-held vibrations is regulated in the provision AFS 2005:6/AFS 2019:3 by the Swedish Work Authority [6]. According to the provision the employer should provide medical check-ups, perform individual risk assessments to define the vibration exposure level, and inform and educate workers about the risk of vibration exposure. However, patients keep telling a different story about their workplace, e.g., in a questionnaire 60% of carpenters claimed to never or seldom have had such information [7].

The European Union Directive 2002/44/EC defines a daily vibration exposure limit value of 5.0 m/s ² and an action limit value of 2.5 m/s² to protect workers from injuries [5]. According to a risk nomogram 10% of exposed workers will develop injuries after a certain time depending on exposure level [8]. The calculations are based on frequency-weighted acceleration values and are stated in the standard ISO 5349-1 [9]. Of all patients referred for hand-arm vibration injury to Occupational and Environmental Medicine (OEM) in Lund 2012-2015, one third had an estimated exposure above the limit value and almost half (47%) above the action value. Furthermore, targeted inspections from the Swedish Work Authority, showed

deficiencies in the sentinel health surveillance and risk assessments in 70% of the 600 inspected workplaces [10]. In a follow-up survey on patients diagnosed with hand-arm vibration injury at the Swedish OEM clinics, of 246 patients less than one fifth (18%) reported that the employer had organized medical check-ups, and only 8% that they had received information about the risks of vibration exposure [11].

*Diagnosis, severity grading and pathophysiology.*

Hand-arm vibration injury entails a neurosensory, a vascular and a musculotendinous component with a diffuse distribution to the finger-hand-arm area. The condition is also referred to as hand-arm vibration syndrome [12, 13]. Correct and timely diagnosis and severity grading of injury are crucial for preventing progression to manifest injury. There is an ongoing debate on how to grade the severity. Diagnosis and grading rely on subjective reporting of symptoms and a combination of semiobjective sensory modality tests. Different theories about the site of injury have been proposed and studied in both epidemiological and experimentally designed studies. Extended knowledge about pathophysiological mechanism in relation to site specific injury will help to develop targeted therapies, e.g., how to define and take count for individual susceptibility, and to develop pharmacological treatment. Histopathological changes to nerves and blood vessels have been observed [14-18], and several biomarkers involved in the vascular tonus or endothelial cell-lining have been studied [12, 18, 19]. The role of biomarkers specifically associated with the structural changes, e.g., demyelination and axonal degeneration [20] and regeneration [21] and perception of temperature and pain [22] in the hand-arm vibration injury warrants further investigation.

# The clinical assessment

Symptoms in hand-arm vibration injury can structurally originate from:

1) A vascular component with episodic finger blanching, also denoted Raynaud's´ phenomenon (RP).
2) A neurosensory component involving both diffusely distributed neuropathy to finger/hands, and a more localized, proximal nerve-neuropathy wrist, carpal tunnel syndrome (CTS).
3) A third component involving muscles and tendons with occurrence of reduced muscular function and the development of tendinopathies, tenosynovitis, or fibrosis (e.g., Dupuytren´s contracture).

Based on a systematic review and meta-analysis the estimated average effect size for contracting hand-arm vibration injury was on average 4–5-fold, corresponding to a 7.4 OR for neurosensory injury, 6.9 for RP, and 2.9. for CTS [4]. The diagnosis and grading of severity in hand-arm vibration injury are based on a) typical symptoms and clinical findings, b) ruling out other medical conditions and c) sufficient and time related vibration exposure.

## The vascular component

Over hundred years ago adverse health manifestations in the hands were described by Loriga among Italian workers using pneumatic tools [23] and then by Dr. Alice Hamilton, a pioneer in occupational medicine [24]. Dr Hamilton studied a condition that she called "dead man's hands", among stonecutters in Bedford, Illinois. The condition had been defined in 1886 by the French physician Dr. Maurice Raynaud as episodic attacks of finger blanching, Raynaud's´ phenomenon (RP) [25] (Figure 1). Dr Hamilton reported a prevalence of over 80% of RP among the workers [26]. Later, in the 80-ties the same "Bedford-study" was repeated showing the same prevalence among workers [27]. In a meta-analysis from 41 articles published between 1978 and 2013, the prevalence of RR varied from 0% - 53% (average 22%) [4]. RP can be primary, without associated disease. Prevalence of primary RP is about 2-15%, the higher prevalence in women due oestrogen sensitivity to peripheral vasoconstricting factors [28]. Cold and stress are two major triggering factors for the vasospastic episodes. The prevalence of primary RP was (11% of men and 14%) in a study conducted in northern Sweden [29].

**Figure 1.** Raynauds phenomenon.Permission to reuse by wicki commons, https://creativecommons.org/licenses/by-sa/4.0/

Previous frost bites are associated with an three-fold odds ratio for RP [30]. The diagnosis vibration induced RP is mainly based on medical history. It is therefore important to consider other medical conditions that need further investigation and treatment (Figure 2), e.g., secondary RP due to connective tissue disorders, metabolic, or infectious disorders, pharmacological treatments, e.g., betablockers, ADHD and migraine medication or carpal tunnel syndrome. RP may precede by years the occurrence of an overt disease [31]. The use of color charts in the clinical assessment can strengthen the diagnosis of RP [32]. Or even better, if the patient can show a picture of the worst episode of finger blanching taken with the mobile.

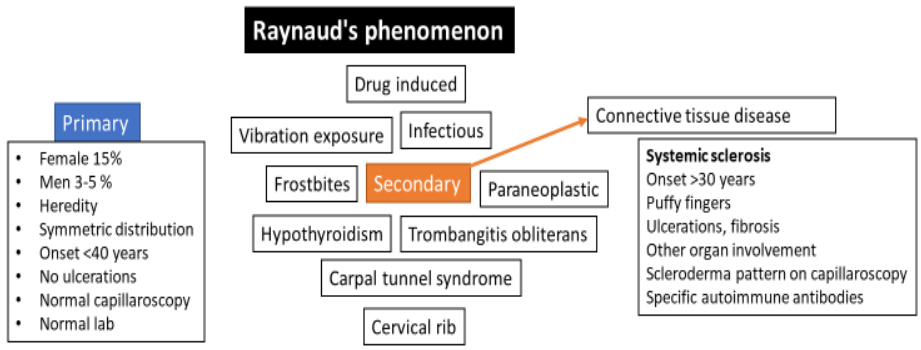



**Figure 2**. Primary and secondary Raynaud´s phenomenon.

A cold provocation test with digital opening pressure or cutaneous temperature plethysmography can be used as a more standardized method in diagnostics. However, these tests will not distinguish a primary RP from a secondary cause [33, 34]. Nail fold capillaroscopy should be performed if suspicion of a secondary RP due to connective tissue disease. Typical morphological changes can be recognized, in systemic sclerosis dilated tortuous capillaries and loss of capillaries are pathognomonic for disease [35-39] (Figure 3).

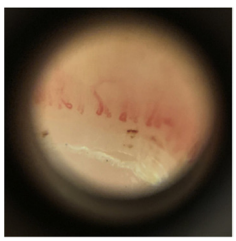


**Figure 3.** Nailfold capillaroscopy captured using a smart phone, showing a typical 'scleroderma pattern' with dilated loops, areas of avascularity and haemorrhages. Reprinted from Best Practice & Research: Clinical Rheumatology, , 2020-02-01, Volume 34, Issue 1, Article 101474, Raynaud´s phenomenon, Herrick A L and Wigley F M,Copyright © 2019 [40] with permission from Elsevier.

Further investigation with duplex MR angiography can be performed to visualize the ulnar and radial artery. Hypothenar hammer syndrome is a differential diagnosis to keep in mind if there is a pathological Allen's test on examination [41, 42].

## Cold intolerance

Cold intolerance or sensitivity has been defined as "a qualitative aberration of cold perception, where cold exposure is perceived as pain (cold allodynia) or discomfort" [43]. It occurs with or without discoloration, numbness, weakness, or stiffness of the hand and fingers, but with no observable vasospasm [44, 45]. Cold intolerance is frequently seen in patients with traumatic hand injuries [46, 47], prevalence in patients after hand fractures were reported to 38% [48] and in combined nerve injury 70% [49]. The symptom is also associated with carpal tunnel syndrome and diabetes mellitus [50]. Based on questionnaires the prevalence in the general population in northern Sweden cold sensitivity was shown to be about 10% for men and 14% for women [29]. Risk factors for cold sensitivity in the hands were among women frostbites, rheumatic disease, migraine, and cold exposure and among men frostbites and exposure to hand-arm vibration [51]. The clinical assessment is based on symptoms, and it can be difficult to distinguish from other symptoms in hand-arm vibration injury, especially RP. The Cold Intolerance Symptom Severity (CISS) questionnaire contain multiple questions on the impact of cold intolerance in the patient's daily life and calculate a sum score [52, 53].

## The neurosensory component

The neurosensory symptoms were until mid-1900s regarded as early signs of vascular injury. Then studies started to look at neurosensory symptoms separately. In a meta-analysis articles from 1978 to 2013 showed a variation in prevalence of neurosensory injury between 17% to 79%, with an average of 43% [4]. The neurosensory manifestations entail symptoms emerging from impairment/injury in *sensory unit*, i.e., sensory afferent nerve fibers and its receptors in the skin. Symptoms can be divided into negative manifestations: impaired perception of touch, temperature, or vibration, impaired finger dexterity or fine motor skills, or impaired grip strength, positive manifestations: numbness and tingling or provocable manifestations: cold intolerance [54].

*The peripheral nerve*

A peripheral nerve can include afferent sensory fibres, leading input from the receptors in the skin to the brain, efferent motoric, terminating at the neuromuscular junction in the muscle or efferent autonomic sympathetic or parasympathetic. Myelinated nerve fibres conduct signals more rapidly than unmyelinated, e.g., the perception of cold is predominately registered by small Aδ-fibres, while the perception of warmth by small, unmyelinated C-fibres (Table 1) [55].

*Mechanoreceptors*

The sensory nerves terminate in the skin as free nerve endings or in specialized receptors. Mechanoreceptors in the skin mediate the detection of touch, pressure, and vibration. There are two slow-adapting types (SAI and SAII) and two fast adapting types (FAI and FAII). The FAI respond to perception of touch (Meissner). The FAII registers vibration, most sensitive at 100-300 Hz (Pacinian). The SAI receptors are associated with Merkel discs and reacts to light touch. Merkel cells express Piezo2, a mechanosensitive ion channel. Merkel nerve endings register deep static touch such as shapes and edges. The SAII reacts to skin stretch (Ruffini corpuscle) [56] (Figure 4, Table 1).

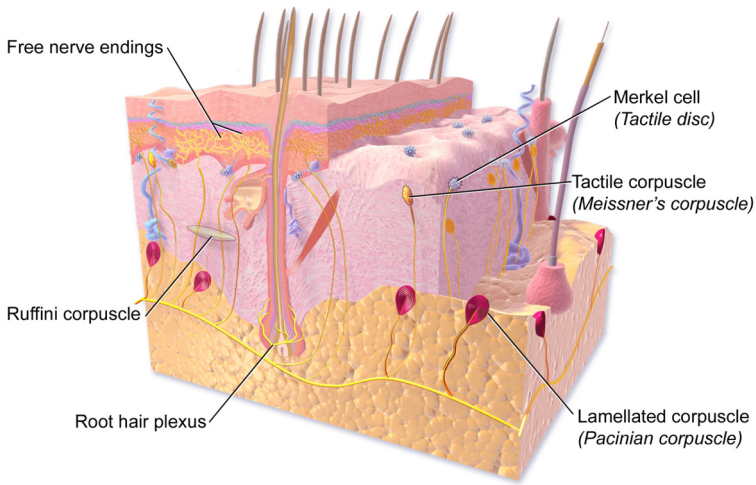



**Figure 4.** Mechanoreceptors in the skin. Permisson to reuse: Blausen.com staff (29 August 2014). "Medical gallery of Blausen Medical 2014". WikiJournal of Medicine 1 (2). doi:0. Wikidata Q44276831. ISSN 2002-4436.

*Thermoreceptors*

TRP channels are a type of ion channels permeable to cations, e.g., Ca2+, Mg2+, Na+, K+ and are found in many different cell types [57]. TRP channels give rise to the thermal sensation and help regulate body temperature. TRPV1 reacts to temperatures above 43 ºC, both are expressed on nociceptive primary afferent Aδ and C-fibres in humans and can be activated by capsaicin giving rise to burning pain. TRPA1 is activated at low temperatures (<18 °C), causing a sensation of painful cold. TRPM8 is activated at temperatures below 25 °C and can be stimulated by menthol [22] (Figure 5).

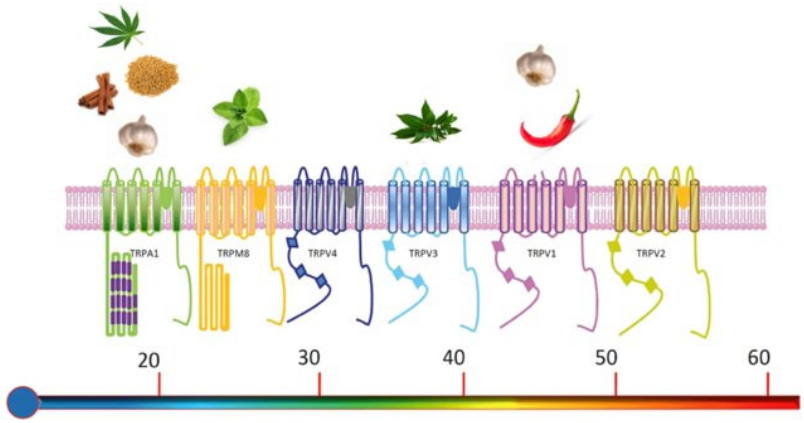



**Figure 5**. Mammalian thermo TRP-channels. Permisson to reuse via license: CC BY-NC-ND 4.0.

**Table 1.** Characteristics of different sensory units

| Nerve fibre | Diameter (μm) | Speed (m/s) | Myelination | Receptor |
|---|---|---|---|---|
| Aα | 12-20 | 72-120 | thick | Proprioceptors<br>Muscle spindle<br>Golgi tendon organ |
| Aβ | 6-12 | 35-75 | medium | Pacinian corpuscle<br>Meissner corpuscle<br>Merkel discs (Piezo2)<br>Ruffini corpuscle |
| Aδ | 1-6 | 4-36 | thin | Free nerve endings<br>TRP channels |
| C | 0.2-1.5 | 0.4-2.0 | none | Free nerve ending<br>TRP channels |

*Clinical findings*

It is advised to perform a test battery of different neurosensory modality tests to best capture the diffuse peripheral neuropathy in patients with hand-arm vibration injury [58-62]. No single sensorineural test method were shown to best correlate to the severity grading scales [63, 64]. An estimations 10 000 vibration exposed workers in Sweden with early injury would pass the medical checkup undetected if using two sensory modality tests instead of four [65]. *Quantitative sensory testing (QST)* is a semiobjective (psychophysical) way of measuring the sensory input registered by the patient [66, 67]. The ability to perceive vibrations, i.e., vibrotactile sense, is dependent on the function of cutaneous receptors and large-diameter (Aβ) afferent nerves. Vibration perception threshold (VPT) at multiple frequencies is used as a diagnostic tool in diabetic neuropathy and in hand-arm vibration injuries Accessed at seven different frequencies there was an increased sensitivity in detecting neuropathies compared to when a single frequency was used [68]. Assessment of perception of touch is performed with Semmes-Weinstein monofilaments® [69]. RollTemp® 25°C and 40°C instrument can be used for qualitative assessment of the ability to discriminate between cold and warmth [70] (Figure 6). However, for more standardized values, quantification with temperature threshold is recommended [71].

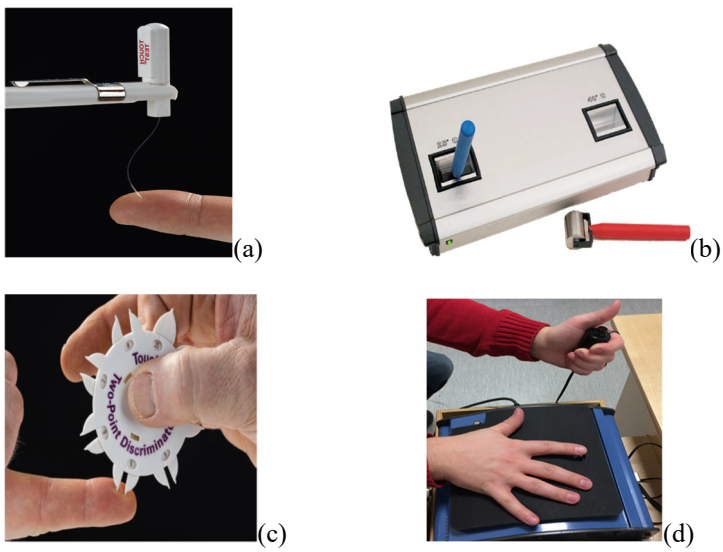



**Figure 6**.(a) Semmes-Weinsteins Monofilament (b) RollTemp® (c) 2PD with Touch Test® (d) Vibration perception thresholds. (a-c) con permisson from Thor Nilsson (d) private foto.

Among the different neurosensory tests VPT scored the greatest deviance, followed by Semmes Weinsteins monofilament and then thermal perception (Figure 7).

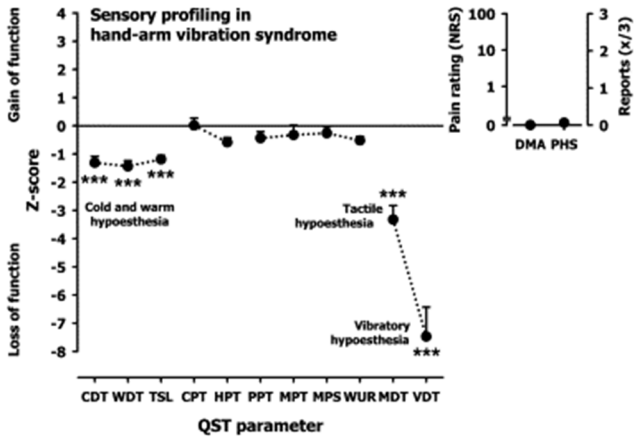



**Figure 7.** HAVS patients showed markedly increased vibration detection thresholds compared to healthy controls using a custom-made vibrameter (stimulus frequencies: 2–600 Hz). Stars denote the level of significance: *** p < 0.001; unpaired t -tests, Bonferroni-corrected for multiple comparisons. +++ p < 0.001; ANCOVA ( F = 169). Rolke R et al. Hand-arm vibration syndrome: clinical characteristics, conventional electrophysiology and quantitative sensory testing. Clin Neurophysiol. 2013. Permisson from Elsevier.

The greatest deviance for VPT was shown at 125 Hz, implying injury to nerves and the corresponding mechanoreceptors, the Pacinian corpuscles. [60, 72] (Figure 8).

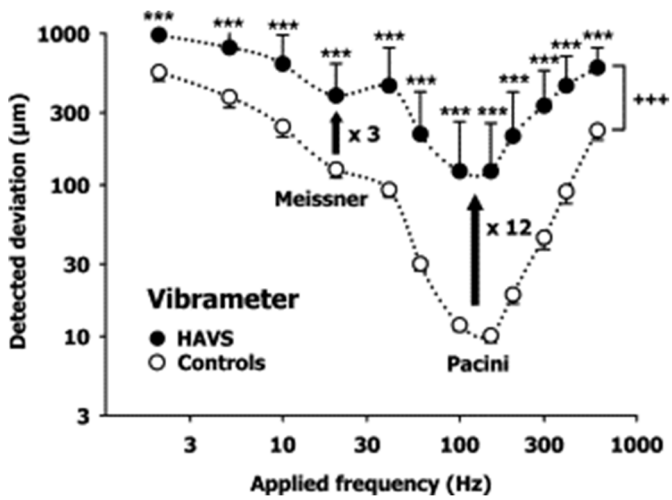



**Figure 8.** *Z* -score sensory profiling in HAVS (filled circles) using quantitative sensory testing (QST). Dynamic mechanical allodynia (DMA) and paradoxical heat sensations (PHS) are presented as QST raw data. Thermal detection thresholds (CDT, WDT, TSL) as well as mechanical detection thresholds (MDT, VDT) were increased (negative *Z* -scores = loss of function). Stars denote the level of significance: *** *p* < 0.001; unpaired *t* -tests, Bonferroni-corrected for multiple comparisons. Rolke R et al. Hand-arm vibration syndrome: clinical characteristics, conventional electrophysiology and quantitative sensory testing. Clin Neurophysiol. 2013. Permisson from Elsevier.

Patients with hand-arm vibration injury also experience impaired dexterity and fine motor skills. These symptoms in conjunction with clinical impairment on testing static two-point-discrimination (2PD) and manual dexterity with Purdue Pegboard are recognized as more advanced levels of injury [71]. For tactile gnosis, a static-two-point discriminator can be used, e.g., Touch Test® Two-Point Discriminator or Dellon-McKinnon® Disk-Criminator [73] (Fig 6). The clinical use of static two-point discrimination (2PD) to screen for impaired tactile gnosis as a way of detecting early injury has been questioned, since impaired 2PD is claimed to appear late, involving both nerve fiber density and the neurosensory input to the sensory cortex in the brain [68, 74]. For manipulative dexterity Purdue Pegboard is recommended [71]. Impaired performance in the Purdue Pegboard test must however be interpreted with consideration of the patient's vision, ability to concentrate, and a central nervous or impairment in the musculoskeletal system must be ruled out. A Jamar dynamometer is recommended for evaluating grip strength [75]. Hand-arm vibration injured patients often report impaired grip strength and poor performance with grip strength test, although at the exterior there is no sign of muscle hypotrophy in the hands, in contrast to longstanding nerve entrapment. Animal studies have shown structural changes in exposed muscle tissue and clinical studies have verified impaired grip strength in vibration exposed patients [76-78].

## Nerve entrapment neuropathies

In addition to vibration, manual handling of vibrating tools in non-neutral wrist positions are occupational risk factors for *carpal tunnel syndrome (CTS)*. The use of vibrating tools is a well-known risk factor for CTS [79, 80]. In a systematic review and meta-analysis showed an effect size of OR 2.9 comparing workers exposed to vibration from hand-held tools with a non-exposed group [4]. The prevalence of CTS in the adult general population is about in 5% in women and 2% in men [81]. Peripheral nerve entrapments at wrist or elbow or plexus level should be tested for (e.g., Phalen's, Tinel's, Roos test) [75]. If positive nerve entrapment tests, further clinical assessment can include neurography and referral to a specialist in hand surgery for evaluation. A generalized polyneuropathy must be ruled out by checking sensory perception in the feet. Blood samples to screen for metabolic diseases, alcohol abuse, vitamin deficiencies, infectious diseases, autoimmune diseases, etc. Examination of the upper extremity including neck and shoulder area to check for radiculopathy, or compressive/focal lesions or other musculotendinous affections. Magnetic resonance tomography can be indicated or electroneurography (ENeG). Symptoms from a diffuse distal neuropathy are however often difficult to distinguish from a peripheral nerve entrapment at the wrist level. Entrapment distally is believed to induce altered biochemical milieu also more proximally, by axonal transport mechanisms (double crush phenomenon) [82]. Increased pressure in the carpal tunnel seems to also affect the ulnar nerve in the Guyon's canal. In a study patients with CTS were shown to have significantly higher VPTs in the finger

pulps of both the index finger and the little finger than healthy controls [83]. ENeG is for certain an objective method, but it only measures the conduction of large, myelinated Aα and Aβ nerve fibers and is inadequate for evaluating small fiber neuropathy [84].

## Musculo-tendinopathies

Exposure to handheld vibrating tools also implies a high ergonomic workload with an increased risk for musculotendinous and musculoskeletal diagnoses in the upper extremities [85-87]. A systematic review finds a doubled risk for *Dupuytren´s contracture* among vibration exposed individuals compared to non-exposed, and between high and low exposed [4, 88]. There is also an established association with *lateral epicondylitis* [89].

## Osteoarthritis

There are studies showing conflicting result regarding an association with skeletal involvement, e.g., with Kienböcks disease [90]. However, a recent meta-analysis shows no consensus regarding an association between radiological findings of arthrosis in finger-hand joints and vibration exposure [91].

## Dose-response

The European Union Directive 2002/44/EC defines a daily vibration *exposure limit* value of 5.0 m/s ² and an *action limit value* of 2.5 m/s² to protect workers from injuries [5]. The vibration magnitude is the root-mean-square, frequency-weighted acceleration, expressed in meters per second squared ($m/s^2$), according to the ISO standard [9, 92]. The health risk associated with vibration exposure is related to the weighted acceleration magnitude during one working day. Job titles with very high magnitude of vibration exposure are e.g., concrete workers, demolition workers, stone cutters and can easily exceed both action and limit values by far. In addition, the harmful effect from impact tools and high-frequency tools are no included in the calculations. Impact tools are in fact shown to be especially harming to blood vessels and nerve tissue [93-102]. However, the present standard give more importance to frequencies between 8 and 16 Hz, thus underestimating the risk [93, 103] (Figure 9). The scientific background for the weighting is based on a few experimental studies of sensitivity thresholds corresponding to sensation response (Miwa,1967). While experimental studies have shown that frequencies around 100-300 which corresponds to the resonance frequency of the human finger [104], causes most harm since the frequency generated in the exposed tissue magnifies [105]. A risk estimation model suggests that an average 10% of exposed workers will develop RP at action limit level after 12.5 years and the sensorineural manifestations to appear after 5.5 years (Figure 10) [106]. Thus, at equal exposures, the neurosensory injury precedes with a 3-time factor shorter latency than RP. In a longitudinal study it was shown that after 20 years of vibration exposure 40% car mechanics had developed neurosensory symptoms, while 25% RP [107].

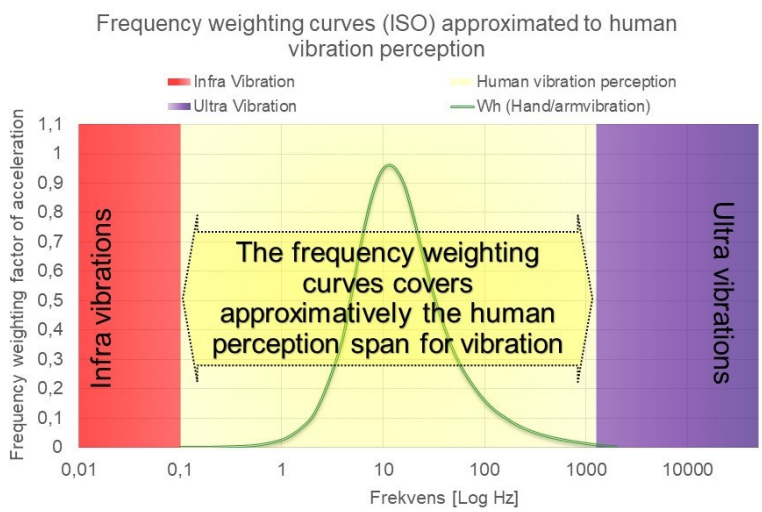



**Figure 9.** The frequency weighting curve (ISO 5349) approximated to human vibration perception. Figure from Jakob Riddar con permisson to reuse.

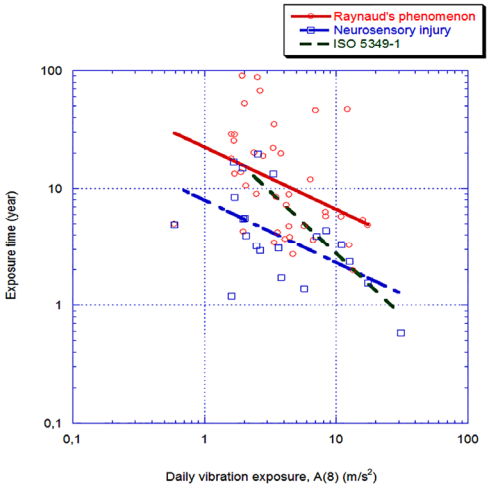



**Figure 10**. Calculated 10% correlation between the prevalence of Raynaud phenomenon and neurosensory injury as a function of the 8-hour equivalent frequency-weighted acceleration and number of years of exposure. Nilsson T et al. Hand-arm vibration and the risk of vascular and neurological diseases—A systematic review and meta-analysis, PLOS ONE 2017 [4]. Permisson by © Open access.

The frequency of vibration is important for the transmission into the tissues, while higher frequencies remain in fingers, lower frequencies proceed proximal to the hands (Figure 11). Grip force is also important for estimating the harmful effect. The effect on blood vessels on vibration and force applied have been studied [108]. In addition, working in cold environment is a known risk factor [29, 30]. For correct risk estimates it is also important to address individual susceptibility. Comorbidity, e.g., polyneuropathy, can influence the susceptibility to vibration exposure. Individuals with diabetes neuropathy, have shown to be more vulnerable to peripheral nerve compression [20]. Women have higher predisposition of primary RP [31], use of nicotine and medication with substances that have vasoconstricting effects, e.g., beta-blockers, CGRP antagonist, ADHD medication might reveal symptoms of RP earlier than the predicted risk.

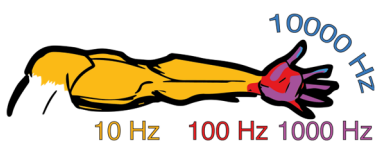



**Figure 11.** Showing the transmission of energy according to frequency. Figure from Jakob Riddar with permission to reuse.

# Severity grading

Grading the severity of vibration injury for clinical work, research and medico legal purposes has been used since the 70-ties. Correct grading is crucial for further medical advice and prognosis. In some countries it is also mandatory to reach stage 2 to be granted worker´s compensation claims [109]. In 1968 the Taylor-Pelmear scale was introduced. It combined the vascular and neural components with seasonal and disability aspects [110]. By time emerging epidemiology called for a separation of the vascular and the neurosensory components since they could develop separately, hence *the Stockholm workshop scales (SWS)* appeared (Table 2 a and b). However, the scales have been subject for subjective misinterpretations. The grading of vascular component (SWS V) depends on the patients reporting of the frequency of episodes of finger blanching which can be related to the surrounding temperature. The prevalence of RP is higher in cold climate, and only a few percent prevalence is reported from tropic regions. (Pelmear and Leong 2000). Other criticisms have been the interpretation of diffuse wording e.g., intermittent, persistent, occasional, frequent, and persistent. In addition, it is not clear which sensory modalities that has to be impaired to fulfil the stage-criteria. In the UK, end of the 90-ties, as a result of a large volume of injured miners seeking compensation, a medical assessment process was set up for the litigation claims. The SWS scale was then modified e.g., stage 2 was divided in to an early and late stage [111]. In 2019 it was time for an updated version; *The International Consensus Criteria (ICC)* was the result from a Delphi round by experts in the field (Table 3 a and b). The frequency of finger blanching episodes has been taken out, instead the number of affected phalanges is scored, documented by a photo of an episode. Impaired manual dexterity requires testing with Purdue Pegboard to achieve stage 3N [71].

The grading of severity of the neurosensory component of injury according to both SWS SN and ICC N are based on the symptom numbness and psychophysical tests. Other symptoms e.g., increased cold sensitivity/cold intolerance are however not included in the scale (except for ICC N3 that also includes the symptom impaired dexterity).

## The Stockholm Workshop Scale

**Table 2 a)** SWS Vascular component

| | |
|---|---|
| **V0** | Exposed to vibration but no symptoms. |
| **V1** | Occasional episodes of finger blanching, only involving distal phalanges |
| **V2** | Occasional episodes of finger blanching, only involving distal and proximal phalanges |
| **V3** | Frequent episodes of finger blanching involving all phalanges on most fingers |

**Table 2 b)** SWS Neurosensory component

| | |
|---|---|
| **SN0** | Exposed to vibration but no symptoms |
| **SN1** | Intermittent numbness with or without tingling |
| **SN2** | Intermittent or persistent numbness, reduced sensory perception |
| **SN3** | Intermittent or persistent numbness, reduced tactile discrimination and/or manipulative dexterity |

## The International Consensus Criteria

**Table 3 a)** ICC Vascular component

| | |
|---|---|
| **0V** | No attacks of blanching |
| **1V** | Digit blanching score 1-4 |
| **2V** | Digit blanching score 5-12 |
| **3V** | Digit blanching score >12 |

**Table 3 b**) ICC Neurosensory component

| | |
|---|---|
| **0N** | No numbness or tingling of digits. |
| **1N** | Intermittent numbness or tingling of digits. |
| **2N** | As in stage 1 but with sensory perception loss in at least one digit as evidenced by two or more validated methods such as monofilaments, thermal aesthesiometry and vibrotactile thresholds |
| **3N** | As in stage 2 but with symptoms of impaired dexterity and objective evidence of impaired dexterity by the Purdue pegboard test. |

## Follow up and prognosis

Patients graded with SWS SN 2 or more are in general recommended not to continue working with vibrating tools. However individual factors are also to consider. Worsening of the condition is not expected once the exposure stops, if so, revaluation of the diagnosis should be made. Other medical conditions can co -exist and may make the individual more prone to injury, e.g., diabetes neuropathy and primary RP. Aggravating impairment is not to be expected once the exposure ceases and should imply further medical investigation. Today there is no specific treatment for the diffuse neuropathy besides stopping vibration exposure. Nerve entrapment at the wrist can be relieved by surgical procedure, but symptoms might return or resist if the patient continuous with the same vibration exposure. Factors that can affect the surgical outcome of CTS are involvement of diffuse more distal neuropathy and lack of workplace-interventions. In a meta-analysis work task related interventions were pointed out as the best conservative treatments in CTS [112].

The vascular component, i.e., attack of finger blanching might decrease if triggering factors (cold exposure, stress, β-blocker, vibration exposure) are evited and pharmacological treatment with calcium-blockers for RP might have an effect. A 15-year follow-up study showed that 43% of workers diagnosed with vibration induced RP improved, while 12% deteriorated and there were probably also other factors, e.g., comorbidity influencing the prognosis [113]. Another longitudinal study on forestry workers with vibration induced RP, finger-systolic pressure improved in almost 50% of the workers. The improvement in finger systolic pressure was however not reflected in the subjective experience of finger blanching episodes. In addition, continued vibration exposure, smoking, other circulatory diseases, and low age at the time of diagnosis had an unfavourable influence on the prognosis [114, 115]. The neurosensory component of injury is considered to have a poor prognosis once manifest. In a questionary based study (without clinical investigation) there was an approximately 30% improvement in neurosensory symptoms after 8.5 years [116]. In a 22-yeas follow up there was a tendency towards irreversibility in hand numbness and finger pain [117]. Recovery from the symptom cold intolerance was much poorer among individuals with vibration injury than among individuals with other nerve injuries [46, 118]. Cold intolerance is associated with a negative impact on quality of life. Desensitization programs as a treatment have been studied after peripheral nerve and brachial plexus injury [119].

# Pathophysiological mechanisms

Initially most epidemiological studies on vibration exposed workers have focused on the vascular component of injury, later on the interest of focus switched to the neurosensory component of injury. Hand-arm vibration injury have also over the years been referred to as a syndrome, HAVS. However, increased insight into mechanisms of injury, have led to adoption of more organ specific diagnoses [120], e.g., small- or large fiber neuropathy, carpal tunnel syndrome or Raynaud's phenomenon.

## Raynaud's phenomenon

The initial vasospasm results in blanching of the skin, followed by cyanosis due to deoxygenation of the static venous blood. When blood returns there is usually a reactive hyperemia. The classical 'triphasic color change' however occurs in about one-third of primary RP patients and two thirds of secondary RP associated with systemic sclerosis. According to consensus from the European society of vascular medicine (ESVMD, blanching alone also allows for the diagnosis of RP [121]. The color changes start at the tip of the finger and spread to more phalanges. Vasospasm may also affect other extremities e. g. nose and ears, and may be associated with other vasospastic disorders, e.g., migraine.

*Primary RP*

The pathophysiology of RP has been suggested to include both structural changes and dysfunction of the blood vessel wall with loss of the control of the vascular tone. Disruption in the balance between vasoconstriction and vasodilation in favor of vasoconstriction results in RP. This can occur via downregulated vasodilation or increased vasoconstriction where both the endothelial cells and neuronal innervation of the smooth muscles can be involved. Individuals with RP shows a more prominent reaction of the arterio-venous anastomoses in the finger vasculature [122]. Primary RP is more common among female, 15-20% compared to men 3-5%. Estrogen increases the expression of cold-sensitive $\alpha_{2C}$-adrenoceptors which augment the cold-mediated constriction in cutaneous arteries. The increased sensitivity of the cutaneous circulation to local cold exposure has been related to mid-luteal phase of the menstrual cycle when estrogen levels peak [40]. Nitric oxide synthases (NOSs) are a family of enzymes catalyzing the production of nitric oxide (NO) from L-arginine. A genetic predisposition to primary RP with the nitric oxide synthase-1-gene (NOS1) involved has been suggested [123]. In a prospective follow up-study, blood levels of Willebrand factor (vWf) was higher at base line in individuals with RP that subsequently were diagnosed with systemic sclerosis [124].

*Secondary RP*

RP is one of the 'red flags' of "Very Early Diagnosis of systemic sclerosis" the others are systemic sclerosis-specific autoimmune antibodies, scleroderma pattern on capillaroscopy and puffy fingers [125, 126]. RP is present in 90-95% of patients with systemic sclerosis. A prospective study showed that in patients with RP that showed scleroderma pattern on capillaroscopy in combination with systemic sclerosis-specific antibodies, 65.9% were diagnosed with systemic sclerosis after 5 years, while 1-3% of patients with RP and normal capillaroscopy and antibody titters, developed systemic sclerosis at 5 years follow-up [127, 128]. A maladaptive production of autoantibodies and cell-mediated autoimmunity, leads to endothelial injury, with diminished blood flow, leading to disturbed angiogenesis, extracellular matrix accumulation and fibrosis in skin and internal organs [129]. Several serological biomarkers have been proposed to be involved in the vasculopathy e.g., endothelin-1 (Et-1), vascular growth factor (VEGF), intracellullar adhesion molecule 1 (ICAM-1), matrix proteins [130-132]. Altered nitric oxide metabolism is suggested to play a pivotal and critical role. Increased NO drives cutaneous fibrosis through oxidative stress, while decreased NO leads to vasoconstriction. Asymmetric dimethylarginine (ADMA), is a competitive inhibitor of nitric oxide synthase (NOS). Treatment with serum l-arginine to lower ADMA has been suggested in patients with scleroderma-RP, while the same beneficial effect were not shown in primary RP [133]. Typical scleroderma pattern in nailfold capillaroscopy are loss of capillaries and compensator vessel growth with dilated mega capillaries [134]

*Vibration induced RP*

It is however not clear whether the pathophysiological mechanism of vibration induced RP resembles that in primary RP or secondary RP or have other mechanisms involved. Subtle changes of nailfold capillaroscopy has been documented in vibration injured individuals [13] and in subjects with diabetes [14]. Different theories for the vascular manifestations have been proposed involving both structural changes and local dysfunction to the peripheral blood vessel wall, and a systemic involvement of the autonomic nervous system [135]. Endothelial injury or dysfunction can be caused by e.g., mechanical (physical) forces; biochemical factors, i.e., overproduction of oxygen-derived free radicals or by, tobacco smoke. Increased smooth muscle thickness, vacuole formation and reduced free available NO have been studied in a rat tail model [96]. Endothelial dysfunction with involvement of nitric oxide synthetase (NOS) and increased free radicals e.g., ROS [136] and inflammatory processes via oxidative stress have been suggested [135, 137, 138]. Reduced luminal radius size, in relation to continuous and intermittent vibration exposure were studied in a rat tail model [17] (Figure 12). A reduced blood flow was observed also in the non-exposed hand under vibration exposure in exposed individuals [139]. Since the reduction in blood flow was significantly greater in the exposed hand, the theory was that direct exposure to

vibration leads to a systemically induced vasoconstriction. Autonomic disbalance has been proposed with an increase in sympathetic activity and/or parasympathetic depression [140-143]. Different studies have shown e.g., an excessive sympathetic activity [144], with increased cardiac sympathetic tone and digital vascular reactivity on cold exposure in vibration-exposed workers [145] and a decreased in parasympathetic cardiac autonomic modulation during vibration exposure [146]. The amount of norepinephrine was significantly increased in vibration injured patients compared to controls after exposure [147] and there was a change in thyroid hormones in individuals with vibration injury in response to cold exposure [148]. Autonomic nervous dysfunction with heart rate varaibility have been shown in in vibration exposed individuals [149, 150], as well as in individuals with primary RP [151] .

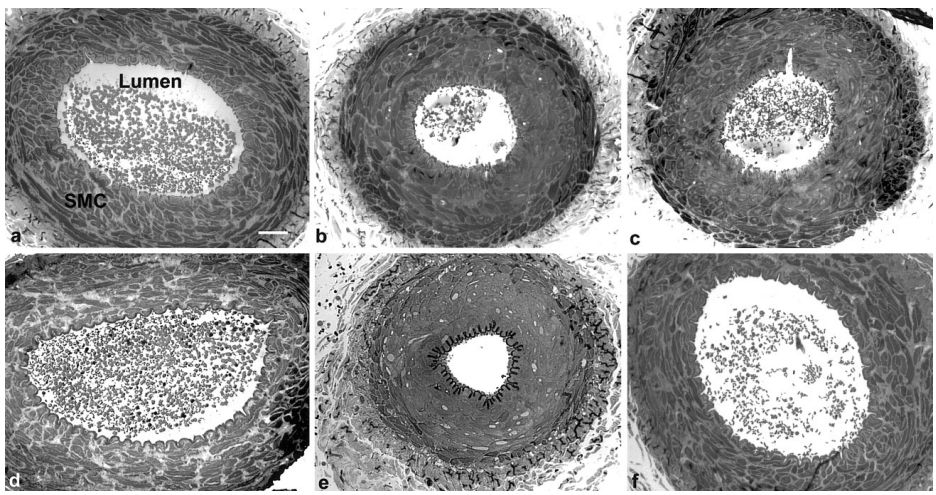



**Figure 12**. Semithin cross-sections of ventral-tail arteries stained with toluidine blue. Compared to the nonvibrated sham (a), the lumen size is smaller for the continuously vibrated rats processed immediately (b) and 24 h after vibration (c). Arteries vibrated intermittently and sampled either immediately (illustrated) or 24 h later do not have smaller lumens (d). Epinephrine causes a decrease in lumen size immediately after application (e). The arteries are dilated 24 h after epinephrine application (f). SMC, smooth-muscle cells. Bar in a is 40 μm for all panels.


## Shear stress, inflammation, and vascular remodelling

The blood vessel walls are constantly exposed to shear stress and cyclic stretch by hemodynamic forces, physiologically generated by the vascular tone, blood viscosity and cardiac output. A certain amount of physiologically generated shear stress and stretch to the vessel wall maintains the vascular homeostasis by regulating vascular remodeling, angiogenesis, and endothelial barrier function [152, 153].

Under pathological conditions, i.e., hypertension, inflammation, or acute injury, oxidative stress signals, e.g., ROS may trigger events that further increase smooth muscle contraction, vascular remodeling, and endothelial barrier dysfunction [154, 155]. Changes in cell morphology, cell function and gene expression have been observed in endothelial cells upon mechanical cyclic strain caused by shear stress and cyclic stretch [152, 154]. *Intercellular adhesion molecule 1 (ICAM-1)* is a transmembrane protein that is being upregulates under conditions of stress or injury. It enhances the inflammatory response by attracting and facilitating the migration of inflammatory cells from the blood stream into the injured tissue [156, 157]. Altered serum levels of ICAM-1 have been associated with connective tissue diseases [158] and correlated to disease activity in patients with RP due to scleroderma [159]. Elevated plasma levels of ICAM-1 have also been shown in an experimental study in vibration exposed individuals [160] and in vibration injured patients compared to controls [19]. *Monocyte chemoattractant protein 1 (MCP-1)* is a chemokine, involved in inflammatory processes, attracts, and enhances the expression, migration, and infiltration of inflammatory cells e.g., monocytes, macrophages, or cytokines to the site of inflammation. Changes in MCP-1 levels have been associated with several pathological conditions, e.g., infectious diseases, connective tissue diseases, cancers, neuroinflammatory diseases, cardiovascular diseases [157]. *Vascular endothelial growth factor (VEGF), basic-fibroblast growth factor (b-FGF) and hepatocyte growth factor (HGF)* are growth factors involved in matrix regulation and angiogenesis, tumorigenesis, and tissue regeneration. They have e.g., been associated with vascular injury and angiogenesis in patients with secondary RP due to connective tissue disease [161]. Serum levels of VEGF and HGF were shown to be related to connective tissue disease activity [162]. HGF, b-FGF and VEGF has shown to significantly increased the growth of vascular endothelial cells [163]. In addition VEGF has also been associated with nerve generation with direct effects on neurons and glial cells, and stimulates their growth, survival and axonal outgrowth and has been implicated a role in nerve degenerating diseases, e.g., diabetes neuropathy [164]. In a rat-tail model diabetic rats showed significant changes in transcript levels for factors involved in synapse formation, peripheral nerve remodelling, and inflammation [165]. *Matrix metalloproteinases MMP-1 and MMP-12* are involved in the breakdown of extracellular matrix in normal physiological processes, such as embryonic development, reproduction, and tissue remodeling and play an important role in cell differentiation, angiogenesis, inflammation, and vascular damage. Serum levels of MMP-1 were increased in RP patients compared to scleroderma [166]. Circulating levels of MMP-12 were significantly increased in patients with systemic sclerosis compared to controls [167]. Tissue inhibitor metalloproteinase (TIMP), conducts the balance between production and degradation of extracellular matrix and a study suggest that matrix accumulation in systemic sclerosis is likely to be due to the presence of excess TIMPs rather than decreased MMPs [166].

## Endothelial injury or dysfunction

The endothelial cells can release factors that affect adhesion and activation of circulating factors to regulate contraction, proliferation, and migration of smooth muscle cells. Breakdown of the endothelial barrier, triggers platelet adherence and release of growth factors leading to smooth muscle proliferation. Electron microscopic structural changes in vibration exposed rats has shown injury of endothelial cells, with activated platelets coating to the exposed subendothelial tissue [168] (Figure 13).

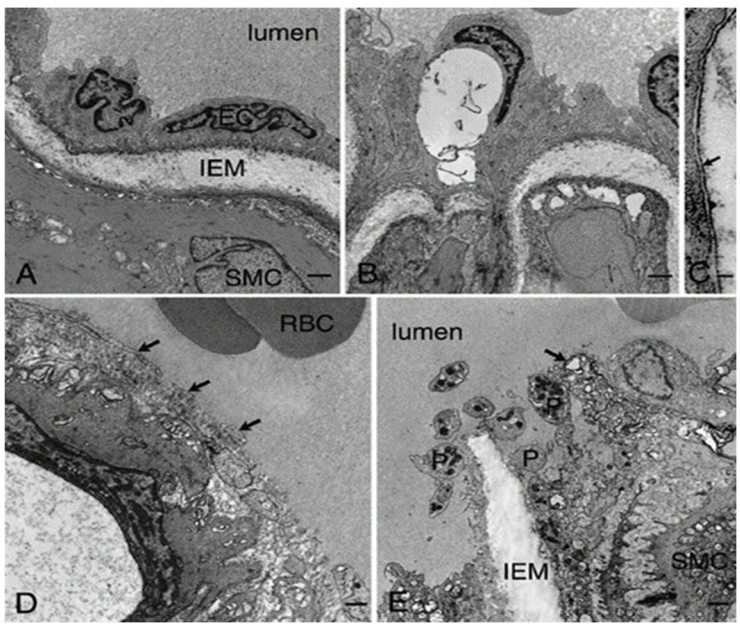



**Figure 13**. **(A)** In the electron micrograph of a non-vibrated artery, a continuous layer of endothelial cells (EC) separates blood in the lumen from the underlying internal elastic membrane (IEM) and smooth muscle cell (SMC).**(B)** Following 1 day of vibration, endothelial cells in the vibrated artery contain large vacuoles not present in controls. **(C)** The vacuoles are limited by double membranes (arrow). **(D)** After 9 days of vibration, the endothelium is discontinuous, and thin processes (arrows) of endothelial cells partially cover the exposed connective tissue (RBC, red blood cells). **(E)** The lesion site shows denuded endothelium, adherence of activated platelets (P) to the exposed connective tissue, a disrupted IEM, and a degenerating endothelial cell (arrow). Bar = 1 μm for (A), 1 μm for (B), 0.05 μm for (C), 0.5 μm for (D), and 0.75 μm for (E).

Curry, B.D., Bain, J.L.W., Yan, J.-G., Zhang, L.L., Yamaguchi, M., Matloub, H.S. and Riley, D.A. (2002), Vibration injury damages arterial endothelial cells. Muscle Nerve, 25: 527-534. https://doi.org/10.1002/mus.10058

*Thrombomodulin (TM)* is a glycoprotein attached to in the endothelial surface. Under normal conditions, TM is present in the blood at low concentrations (<10 μg/L). Elevated levels can be found in conditions associated with endothelial dysfunction, i.e., cardiovascular, inflammatory, infectious, and metabolic diseases. Elevated levels have been studied in vibration injured individuals [19, 169]. TM have been shown in previous studies on vibration injured patients with equal levels as in collagen diseases [170].

*Von Willebrand factor (vWf)* is a glycoprotein involved in the hemostatic pathway, aiding platelet adhesion and aggregation. VWf prolongs the half-life of coagulation factor VIII (Lip, 1997). Elevated levels of vWf have been associated with disease activity in patients with systemic sclerosis [171, 172]. Elevated levels have also been shown in individuals with RP who subsequently developed a connective tissue disease [124]. Association with vibration injury have been studied with varying results [173-176]. Also, other factors can influence blood levels of vWF, e.g., circadian rhythm fluctuation, sex hormones and genetic influence, liver cirrhosis [177, 178].

## Vasoregulation

The vascular tone is balanced by vasoconstricting and vasodilating factors [179]. *Calcitonin gene related peptide (CGRP)* is a potent vasodilator released from perivascular sensory nerve endings of unmyelinated C-fibres and myelinated thin Aδ-fibres [180]. CGRP exerts its effect mostly locally, but studies have shown that serum can be used in biomonitoring, e.g., elevated levels in serum have been shown in individuals in relation to episodes of migraine [181]. Debut of RP have been reported as a side effect of this medication [182]. CGRP induced a neurogenic vasodilation, attracting inflammatory cells. Slower wound healing have been associated with a deficit of CGRP-containing nerves [180]. Skin biopsies have revealed a significant reduction in the number of calcitonin gene-related peptide (CGRP) immunoreactive neurons in patients with primary RP, and secondary RP due to scleroderma compared to controls [183] and also in vibration injured individuals compared to controls [184]. Furthermore, individuals with RP has shown increased sensitivity to intradermal administered *endothelin-1(Et-1)* as a consequence of deficiency of CGRP-containing nerves in the distal digital skin [185]. Et-1 is a potent vasoconstrictor and elevated levels have been found in patients with primary RP [161]. Increased blood- levels of Et-1 have been shown in individuals with RP [186], but in individuals with vibration-induced RP the results have been varying [175, 187-189].

## Cold intolerance

The exact mechanism behand cold intolerance in hand-arm vibration injury is yet not elucidated. Both RP and frostbites are known risk factors. A prospective study showed that “a sensation of cold hands” was a risk factor for later developing RP [190]. Cold intolerance was reported in half of individuals with frostbites and in 60% in patients with non-freezing cold [191]. Besides being a vasoconstrictor, Et-1 is associated with pain, augmenting the effect of nociceptors acting on peripheral nerves and intradermal injection of Et-1 has shown to induce pain and cold hyperalgesia [57]. A study on individuals with cold intolerance due to non-freezing

cold injuries, showed that after a cold provocation test however, individuals with non-freezing cold injuries reported cold intolerance and levels of Et-1 were increased compared to controls, but at baseline there was no difference [192]. Hence, Et-1 seems to be a biomarker best captured by in an experimental setting. The exact mechanism and site of injury is not clears, it may reside in both sensitized sympathetic nerves and/or local injury to endothelial cells. In an experimental animal study, elevated levels of Et-1 were shown in associated with structural changes in blood vessels, e.g., vacuole formation and disruption of the endothelial cell lining [18].

The role of thermoreceptors in cold allodynia is highly interesting. *Transient receptor ankyrin potential-1 TRPA-1* is a TRP-related channel responding to cold temperatures and is co-expressed with TRPV1 on nociceptive primary afferent C-fibres in humans. TRPA1 plays a role in regulating vascular tone in the periphery via a CGRP-mediated mechanism [193]. TRPA1 has been associated with cold intolerance and inflammatory pain [56, 194-196]. Knocking out the TRPA1 gene in mice eliminated avoidance of temperatures below 17 °C and in contrast, the presence of a TRPA1 channel agonist caused increased cold intolerance during mild cooling [197]. NO via ROS targeting TRPA1, co-expressed with TRPV1 and CGRP in small sensory neurons (Aδ and C-fibers) is suggested to be involved in neurogenic inflammation, sensitization and hyperalgesia (in mice) [198] (Figure 14).

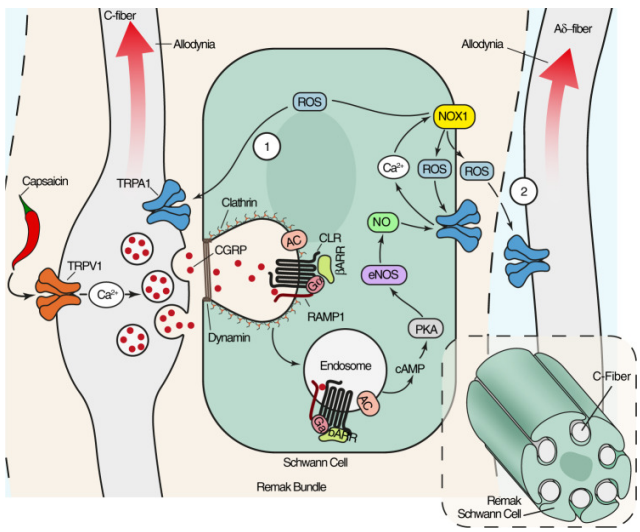



**Figure 14.** Schematic representation of the pathway that signal prolonged cutaneous allodynia elicited by CGRP released and associated with neurogenic inflammation.The pro-migraine neuropeptide, CGRP, released from trigeminal cutaneous afferents, activates CLR/RAMP1 on Schwann cells. CLR/RAMP1 traffics to endosomes, where sustained G protein signaling increases cAMP and stimulates PKA that results in nitric oxide synthase activation. The ensuing release of nitric oxide targets the oxidant-sensitive channel, TRPA1, in Schwann cells, which elicits persistent ROS generation. ROS triggers TRPA1 on adjacent C- (1) or Aδ-fiber (2) afferents resulting in periorbital allodynia, a hallmark of migraine pain. The inset shows several unmyelinated axons invaginated into a Schwann cell forming a Remak bundle. With permission from CC BY4.0 © De Logu et al. 2022. Schwann cell endosome CGRP signals elicit periorbital mechanical allodynia in mice. Nat Commun. 2022 Feb 3;13(1):646.

## The neurosensory component of injury

*Animal studies*

Rat tail is often use in experimental animal models of vibration exposure. The natural frequency of the rat tail is in the range of human finger resonant frequencies (100-350 Hz) [100]. Animal models of vibration-induced injury have shown that vibration results in functional, morphological, and cellular changes e.g., a reduction in nerve fibre density and demyelisation of myelinated nerve fibres [17, 96, 199, 200] (Figure 15). Destruction of the myelin sheath was shown to occurred earlier and more frequent than injury to the axon (rabbit) [201].

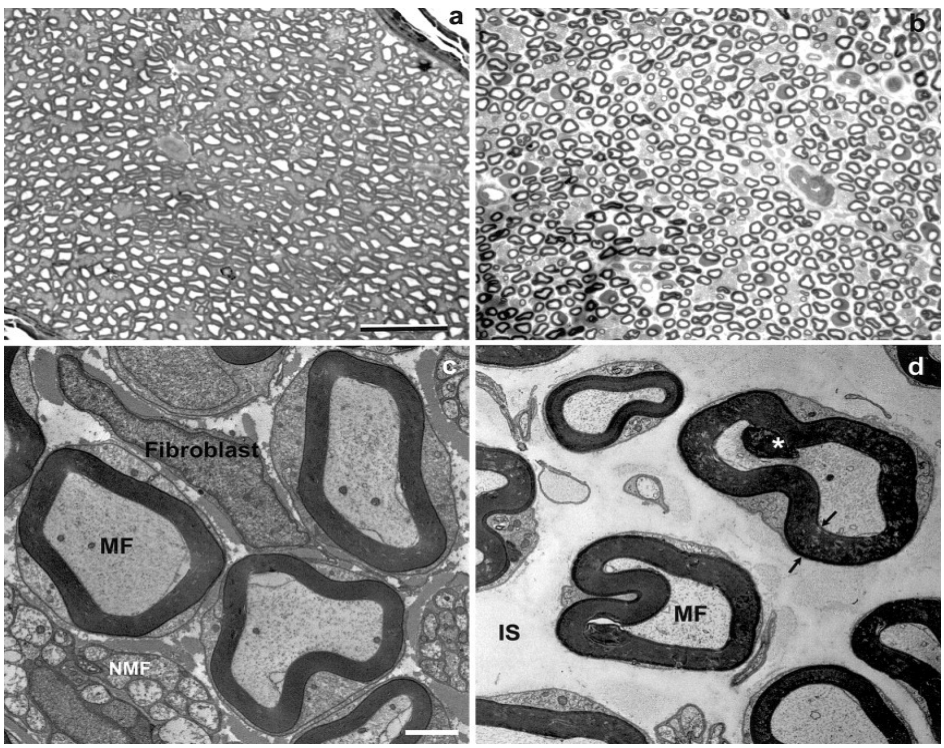



**Figure 15.** Light- and electron-microscopic images of tail nerves. (a) The semithin cross-section of the tail nerve from a sham-vibrated rat demonstrates that the myelin is evenly stained with toluidine blue, and there is very little interstitial space between nerve fibers. (b) Following both patterns of vibration, immediately and 24 h after vibration, the myelin stains darker with toluidine blue and exhibits focal thickening. The interstitial area is enlarged, indicating edema. An example from the intermittent vibration group sampled immediately is illustrated. (c) At the electron-microscopic level, the myelin membranes are compact except for tiny foci of separation in the sham-vibrated control nerves. Myelinated (MF) and nonmyelinated fibers (NMF) are closely packed. (d) Vibrated nerves immediately and 24 h after vibration exhibit larger and more extensive areas of separation of the myelin membranes (arrows), and frequently the myelin sheaths show decompaction (*). There is also increased interstitial space (IS) between fibers when vibrated. An example from the intermittent vibration group sampled immediately is illustrated. Bar in a is 40 μm for a and b; bar in c is 0.5 μm for c and d. Govindaraju, S.R., Curry, B.D., Bain, J.L.W. and Riley, D.A. (2006), Comparison of continuous and intermittent vibration effects on rat-tail artery and nerve. Muscle Nerve, 34: 197-204. https://doi.org/10.1002/mus.20578. Permission to reuse from © 2006 Wiley Periodicals, Inc.

*Human tissue*

Skin biopsies from fingers in patients with vibration injuries have shown Schwann cell activation, an increase in fibroblast cell number, fibrosis, loss of the myelin sheath, and reductions in the elastic membrane and axonal size [14, 15, 184, 199].

Biopsies from the posterior interosseus nerve in patients exposed to vibration showed structural changes with demyelination [16] (Figure 16). These changes may be the mechanism(s) underlying increased susceptibility to nerve entrapment in vibration-exposed individuals, according to light microscopic findings in individuals with diabetes [202], where a higher prevalence of nerve entrapment has been observed [20, 203].

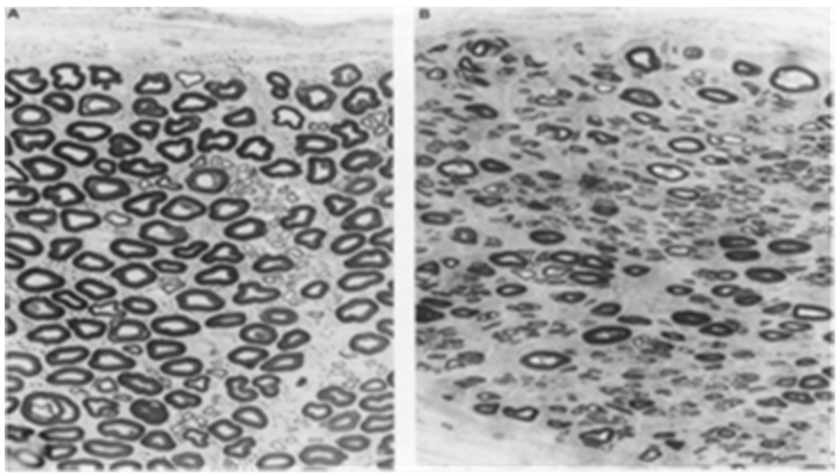


**Figure 16.** Dorsal interosseous nerve: transverse section 05 ,im thick in epon, stained with toluidine blue, showing myelin sheaths as dark structures encircling the axons. In a normal nerve (A), the sheaths are regularly sized and distributed In a nerve from a man exposed to vibration (B), only a few of the myelin sheaths are of normal size and many are small and irregularly distributed in a dense matrix of reactive connective tissue (case 2). Originally x 70. Reproduced from Strömberg et al. Structural nerve changes at wrist level in workersexposed to vibration. Occupational and Environmental Medicine 1997; 54:307-31 13. © 1997, BMJ Publishing Group Ltd. with permission from BMJ Publishing Group Ltd.

*Cortical reorganization*

Also, other pathophysiological mechanisms behind the neurosensory manifestations in hand-arm vibration injury have been proposed e.g., a disturbance in the afferent impulse to the brain leading to impaired muscle function in the hand. The synchronously, repetitive movements as in vibration, have shown to alter area of the receiving input in the brain´s somatosensory cortex [204, 205]. Studies with functional magnetic resonance imaging has shown altered signals in the somatosensory cortex representing the hand [206]. In addition, peripheral neuropathy in sensory nerves led to cerebral reorganization [207-210].

## Nerve injuries and regeneration

Nerve trunks can be subjected to a number of different trauma and diseases, such transection, laceration, vibration exposure, and neuropathy like in diabetes and other metabolic diseases. After a nerve injury, if it is of sufficient severity, the nerve fibers may degenerate distal to the injury (Wallerian degeneration). Nerve degeneration induces a variety of biological processes in the affected nerve, including inflammatory response with macrophage recruitment [21]. The Schwann cells both participate in clearance of the cell debris as well as trigger macrophage recruitment to clear myelin debris and the remaining parts of the axon. If the injury is a nerve transection, a fibrin matrix is formed between the two severed nerve ends that guides the migration of the Schwann cells and the outgrowth of axons across the site of lesion. The environment in the distal nerve end is important to guide a correct nerve regrowth [21]. Similar regeneration processes are going on in different neuropathies during degeneration and regeneration to a various extent. The macrophages also play an important role in guiding Schwann cells along blood vessels and growth factors stimulating angiogenesis, e.g., VEGF-A, are involved to optimize the net effect between nerves and blood vessel distribution [21]. However, regrowth will make the axons wavier, and axon can twist around each other, appearing like "spaghetti (Figure 17).

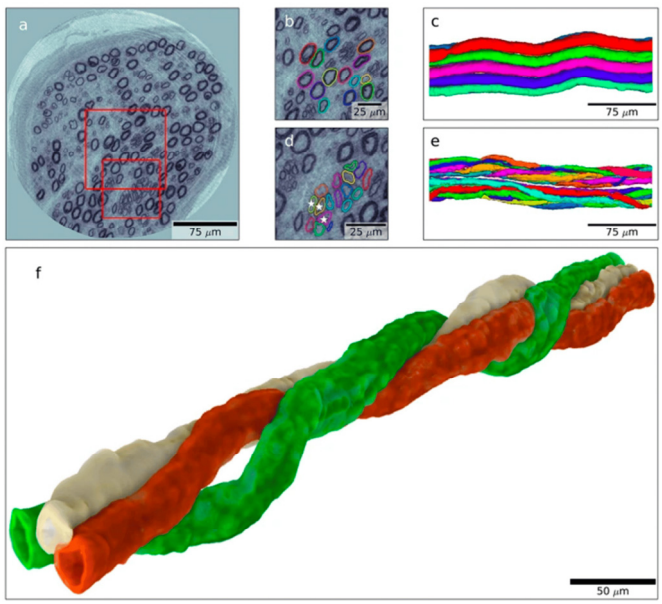



**Figure 17.** Nanotomographic images of a human posterior interosseous nerve from an individual with diabetes. (**a**–**c**) Tomographic slices with enlarged areas used to create 3D images in (**c**) of the segmented nerve fibers marked in (**b**) (healthy nerve fibers with different colors; note wave form). In (**d**), an enlargement of an area marked by small red square in (**a**) is shown with regenerative clusters (i.e., regenerating nerve fibers indicated by different colors) shown in 3D in (**e**). Finally, 3D volume rendering the three axons is marked by stars in (**d**) and are illustrated in (**f**) with one axon (green) twisting around the other two (grey and red). Reproduced by permission from Dahlin et al.(based on and published under a CC BY License: http://creativecommons.org/licenses/by/4.0/.

A variety of factors are involved in the nerve degeneration and regeneration processes. Caspases (cysteine-aspartic proteases) are a family of protease enzymes mediating programmed cell death in a controlled way to maintain homeostasis between degradation and regeneration. *Caspase-3* has been associated with the apoptotic response in Schwann cells to balances the proliferative response of these cells after a nerve injury [21]. Apoptosis is a form of programmed cell death where the cell undergoes morphological changes, to minimize its effect on surrounding cells to avoid inducing an immune response [211]. Overactivation of some caspases, such as caspase-3 can lead to excessive programmed cell death, e.g., in neurodegenerative diseases caspase-3 is an executor and needs caspase-8 for initiation.

*Schwann cells* differentiate to myelinating or non-myelinating Schwann cells (SC). The dedifferentiation process of the Schwann cells after injury can be disturbed by continuous trauma, oxidative stress, metabolic changes, making the environment unfavourable and the machinery may run amok, e.g., Schwann cells fail in differentiating to its right niche (Figure 18). Developing SC and unmyelinated SC express several biomarkers, e.g., glial fibrillar acidic protein (GFAP), a cytoskeletal protein. [212]. GFAP is a proposed marker for axonal injury, associated with several chronic neuropathies in humans [213] and has been detected in nerve biopsies from patients with type 2 diabetes [214]. Non myelinating SC (also called Remark-SC) surrounds many small unmyelinated nerves (Remark bundle). These non-myelinated SC are suggested to play an important role in the development of neuropathic pain both with and without a nerve injury [215]. Experimental studies have shown that altering SC-function can change the outcome of a regeneration process, e.g., loss of a gene resulted in an increased numbers of unmyelinated C-fibers and an increased hypersensitivity to mechanical and thermal stimuli in the absence of injury [216].

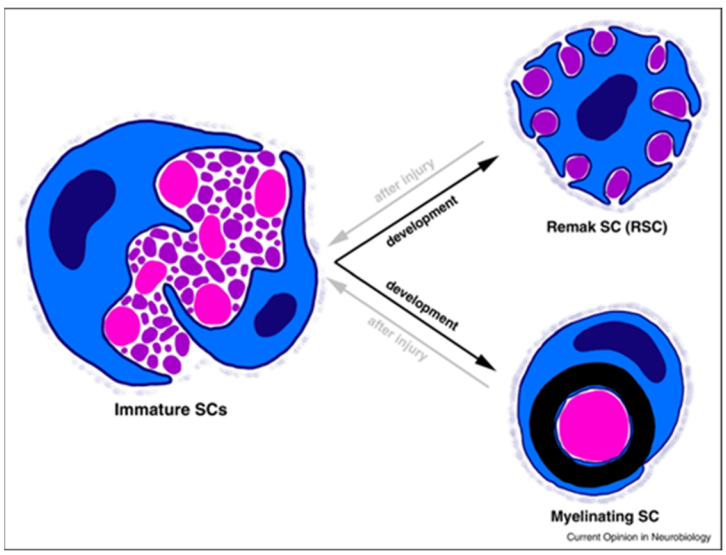



**Figure 18.** After injury (gray arrows) both Remark-SCs and myelinating SCs can dedifferentiate back to an immature state and then re-differentiate to aid in nerve generation. Breanne L Harty, Kelly R Monk, Unwrapping the unappreciated: recent progress in Remak Schwann cell biology, Current Opinion in Neurobiology, Volume 47,2017, Pages 131-137. Permission to reuse from Elsevier.

Nerves can also be compressed, such as in carpal tunnel syndrome (CTS). The pathophysiology in chronic nerve compression can be described as in the schematic illustration below (Figure 19). Hypoperfusion of nerve fibres givie rise to intermittent sensation of tingling and numbness, proceeding to oedema and connective tissue thickening with persistent nubness and abnormal thresholds. Eventually, there is focal and then diffuse demyelination with reduced conduction velocity on ENeG. As axons get affected, the patient presents with reduced grip strenght and atrophy of innervated muscles, 2PD is abnormal and neurography shows a decreased amplitude [217].

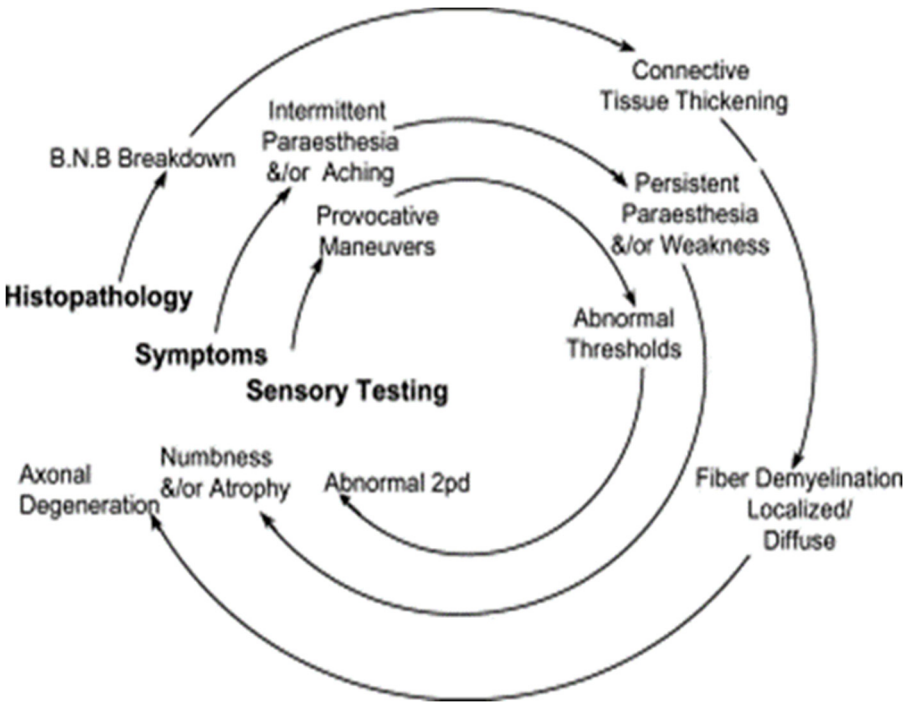



**Figure 19.** Image from Novak and Mackinnon, Evaluation of Nerve Injury and Nerve Compression in the Upper, Journal of Hand Therapy 2005, [218]. Permission from Elsevier.

Chronic mechanical stimulation e.g., shear stress has shown to induce SC demyelination and increased SC apoptosis and proliferation, in absence of evident axonal injury (Gupta and Steward 2003, Belin, Zuloaga et al. 2017). A reduced myelin protein gene expression was shown after shear stress (Gupta, Truong et al. 2005). *Myelin basic protein (MBP)* is a lipid-interacting protein. A change in levels of MBP has been observed in nerve tissue from individuals with diabetes [214]. SC also secrete various neurotrophic factors, that have a protective and nutritional role in neurons [219]. *Neurotrophin-3 (NT-3*) is one growth factor that binds to tyrosine kinase receptors TrkA, TrkB, and TrkC, p75NTR and has been associated with

neuronal survival, axonal outgrowth, synaptic plasticity and neurotransmission. [21, 220]. *Brain derived neurotrophy factor (BDNF*) and *β-nerve growth factor (β-NGF)* are growth factors that regulate nerve function. Increased levels have been detected in association with nerve repair after nerve injury. Upregulation of BDNF and β-NGF were detected in cervical intervertebral discs in rats exposed to whole body vibration [221]. *Galanin* is a neuropeptide with a wide distribution in the nervous system, often co-localization with classical neurotransmitters. Galanin stimulates growth hormone and prolactin secretion and is associated with influence on cognition, feeding, nociception, mood regulation, and neuroendocrine modulation [222]. Upregulation in association with pathological conditions within the nervous system. Elevated levels have been studied after traumatic nerve injuries and has shown an associated with neuropathic pain [223]. In a recent, register based study, elevated levels were shown in individuals with a history of occupational exposure to hand-held vibrating tools, compared to occupationally non-exposed individuals [224].

*Heat shock proteins* are detected in structures under stress [225, 226] to preserve nerve function [227] and to prevent apoptosis [228]. HSP27 is upregulated after nerve injury in both injured nerves and the dorsal root ganglia [229]. Significantly lower serum levels of are observed in patients with diabetes compared to individuals with impaired glucose intolerance and controls. In addition, elevated serum levels of HSP27 are associated with better nerve function and fewer neuropathic signs in all groups [230].

# Aims

Detection of early injury is important, since once manifest there is little or no improvement even if exposure stops. Diagnosis relies on medical history and semiobjective test and grading of the severity by the commonly used grading system is prone to misclassification.

*Paper I - The carpenter study*

- To assess whether carpenters show more signs of adverse health manifestations to their hands compared to painters (assumed to be less exposed to hand-arm vibration).
- To describe the clinical picture of injury and how it presents in young carpenters.

*Paper II - The patient study*

- To assess the concordance between two grading scales, the Stockholm workshop scale (SWS) and the International consensus criteria (ICC) for the neurosensory component in hand-arm vibration injury.
- To describe the clinical picture in patients with hand-arm vibration injury in terms of symptoms, clinical findings, and severity of injury.

*Paper III and IV - The biomarker studies*

- To assess serum biomarkers in patients with hand-arm vibration injury.
- To improve knowledge on pathophysiological mechanisms and to identify objective markers for accurate and timely diagnosis.
- To assess the association between biomarkers and the severity of the vascular and neurosensory injury.

# Methods

## Study design and study population

Paper I is a cross-sectional field study, whereas Paper II, III and IV has a case series design. The study population in paper I consists of carpenters and painters, while Paper II consists of patients diagnosed with hand-arm vibration injury. In paper III and IV a control group has been added for comparison to the patients (Fig 20).

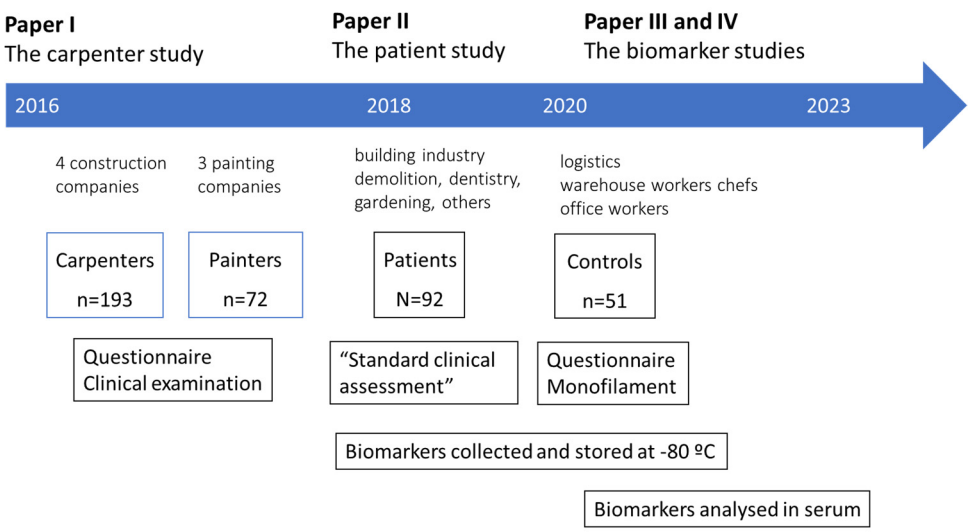



**Figure 20**. Timeline and study participants

*Paper I*

Carpenters working for two medium-sized and two large construction companies were examined at 18 different work sites between April 2016 and April 2018. All the carpenters at these work sites were invited to take part in the study. The painters were recruited from three medium-sized painting companies, and were invited to participate at staff meetings, as they worked at different, smaller work sites. They were examined between May 2018 and September 2019. All 193 invited male carpenters (response rate 100%; one female carpenter was examined but was

excluded from the data analyses). There were 139 male painters at the companies eligible for the study, 108 of whom attended the staff meetings, and were invited to participate in the study. The others could not be contacted. Of these 108, 72 agreed to participate in the study (participation rate 67%). All eight female painters invited to take part in the study participated but were not included in the data analyses.

To assess the generalizability of the results, a shortened questionnaire was sent by post to all the carpenters who worked at the same construction companies, but at other work sites, for three of the four companies included in this study (one company refrained from sharing contact information to workers). This questionnaire was also sent to male carpenters at other construction companies, randomly selected from a list provided from the relevant trade's union. Two reminders were sent. The shortened postal questionnaire was returned by 202 of the 410 male carpenters at the construction companies chosen for the study (response rate 49%), and by 297 of 708 at other companies (response rate 42%). The different groups of male carpenters are visualized in figure 21.

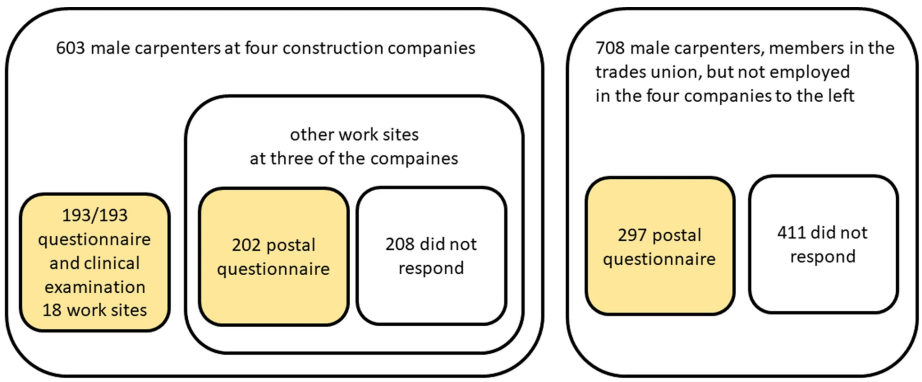



**Figure 21**. The differtent groups of carpenters in the study. Permission by CC BY

*Paper II*

100 patients, consecutively referred to the Occupational and Environmental Medicine Clinic in Lund were asked if they wanted to participate in the study. Exclusion criteria were not having a diagnosis of hand-arm vibration injury (8 individuals). 92 patients (86 men and 6 women) were enrolled from August 2018 until February 2020, in whom 8 patients were no longer exposed to hand-arm vibration at the time of the study.

*Paper III and IV*

To collect blood samples from a control-group all individuals at a number of work sites were invited and included to participate, the only exclusion criteria were a diagnosis of hand-arm vibration injury. From total 64 individuals who accepted to participate, two were excluded since they had symptoms and impaired

monofilament testing in combination with some ongoing vibration exposure, furthermore for 11 individuals where current vibration exposure could be suspected from questionnaire data, were excluded. Finally, 51 controls were included in the statistical analyses with the 92 patients (Figure 20).

## Setting

The participants were instructed not to use nicotine or vibrating tools prior to the clinical examination. Examinations for all study participants were performed in a quiet room at room temperature. Carpenters were examined at their work sites, and painters at their worksites or at the Occupational and Environmental Medicine in Lund. Before starting the tests, the finger skin temperature was measured in the second and fifth fingers bilaterally (Testo 845). If the finger temperature was below 28°C participants were asked to hold warm pads in their hands (patients might also be asked to cycle on a bike) for rewarming.

## Symptoms

*Paper I*

Carpenters and painters were asked to fill in a questionnaire on a laptop (30–45 min). The participants were able to go back and change an answer, but not to skip questions. Some answers generated follow-up questions. An examiner was present in the room to answer any queries. The following questions about neurosensory symptoms from the hand were asked: *"Do you experience: increased sensation of cold; impaired perception of touch; impaired perception of warmth; impaired perception of cold; numbness or tingling when not working with vibrating tools; pain in the fingers/hands when cold; a tendency to drop things; or difficulty buttoning clothes?"* The possible responses were: "Not at all", "Insignificant", "Somewhat" or "Quite a lot". The alternatives "Somewhat" or "Quite a lot" were considered a positive response. If a positive response was given to any of these questions, the participant was asked which of these symptoms that appeared first and when. More than one symptom could be given.

For the vascular manifestations the following question was asked: "*Do one or more of your fingers turn white when you are exposed to cold or dampness?*" A colour photograph was shown to facilitate recognition of the condition. Possible responses were "No" or "Yes". Those who responded "Yes" were considered to have Raynaud's´ phenomenon and were asked to draw the extent of their worst occurrence on a diagram of a hand. They were also asked to state the frequency of finger blanching episodes: "Once a month or less", "Once a week", "Once a day", "Several times a day", "All the time". In addition, they were asked when their symptoms of white fingers started.

*Paper II and III*

The questionnaire included six questions where the response yes or no could be indicated separately for the right and left hand. *Do you experience:*
a) numbness or tingling?

b) numbness or tingling during the night?

c) pain/discomfort in fingers/hands during cold exposure?

d) white fingers when exposed to cold or dampness?

e) poor grip strength?

f) poor fine motor skills or clumsiness?

The questionnaire for Paper I-IV also included questions on age, sex, nicotine use, medication, and concurrent diseases (cardiovascular, diabetes, thyroid, rheumatic). Paper II-IV also a about previous frostbites to hands.

## Clinical findings

*Paper I*

After completing the questionnaire, the participants underwent testing of finger perception of dig II and V bilaterally. Perception of touch was tested with a Semmes Weinstein Monofilament® (detection threshold 3.61, calibrated such that 0.271 g of force is required to bend it when touched on the skin of the finger)[69]. The participant was asked to report when he felt the touch of the filament when it was applied to the finger pulps, once on each finger. For 2PD a static 2PD test with a two-point discriminator (separation 5 mm) was used; seven correct responses out of ten attempts were considered normal. The ability to distinguish between cold and warmth was tested with temperature rolls at 25 °C and 40 °C (RollTemp® instrument), randomly applied to the middle phalanges [70]. Perception of vibration was tested with a VibroSense® equipment. VPT were recorded at 125 Hz and 250 Hz. VPTs exceeding one standard deviation (SD) above the mean value in the age-adjusted reference material were defined as impaired perception of vibration according to standards of the method. If the participant showed increased VPTs in the digits, the lower extremity was examined, by applying vibrations of 128 Hz with a Vibratip® device at the base of the proximal phalange of the big toe. In addition, specific nerve entrapment tests were performed to diagnose nerve entrapment in the neck or upper extremities, including tests of neck mobility, the strength of selected muscles and provocation of the brachial plexus, as well as the median, radial and ulnar nerves.

*Paper II*

Finger-perception was tested in the second and fifth fingers bilaterally. *Perception of touch* was tested with a Semmes Weinstein Monofilament, pocket version, with five filaments for hand testing (0.08–279 g). The patient was asked to report when they felt the touch of the filament, within 3 s after the third application. If the patient was unable to detect stimulation with a filament, No 3.61, corresponding to 0.4 g force, the perception of touch was considered impaired. *Perception of vibration* was tested with an equipment from VibroSense®. Vibration perception thresholds were recorded at seven frequencies from 8 to 500 Hz. Patients with a sensibility index (defined as the ratio between the area under the test curve and that under an age-specific reference curve) below 0.8 (were considered to have impaired vibrotactile sense [231]. For *tactile gnosis* a static two-point discrimination (2PD) test was performed with a Dellon-McKinnon® Disk-Criminator (Touch-Test). If the patient was unable to respond with a separation of 5 mm the test continued with wider spaces, and the 2PD was considered impaired [73]. *Manipulative dexterity* was assessed with a Purdue Pegboard Manual Dexterity test (model 32020A). Patients aged 45 years or younger who scored less than an average of 12 pins in 30 s were considered to have impaired dexterity; the corresponding number for those older than 45 years was 10 pins [71]. *Hand grip strength* was tested with a Jamar hydraulic dynamometer. The test was performed with the right and left hand alternately. The mean value of three attempts was calculated for each hand separately [75]. The *temperature identification* test was performed with temperature rolls at 25°C and 40°C; RollTemp® instrument, in the second and fifth fingers bilaterally. If the patient could not identify if the RollTemp® instrument 25°C was cold and the RollTemp® instrument 40°C was warm, the patient was considered to have impaired identification of warmth or cold [70]. There was no strict instruction on whether the instrument should be applied to the distal phalange or more proximal, nor was the time duration of the skin contact specified. The ability to discriminate between blunt or sharp was tested with both ends of an upholstery needle (pinprick test). There was no strict instruction on how to perform this test.

## Clinical conditions

The following clinical conditions were defined:

*Neurosensory affection*

Paper I: Symptoms reported in the questionnaire were combined with clinical findings to indicate neural manifestation. Neurosensory affection was defined as having at least one symptom in the fingers/hands (impaired perception of touch, warmth, or cold, impaired dexterity, increased sensation of cold, numbness or tingling, or pain in finger/hands when cold) and at least one clinical finding: impaired perception of touch, warmth, cold, or vibration, or impaired 2PD.

*Raynaud's phenomenon*

Paper I-IV: Participants that had answered "yes" on the question if they had episodes of finger blanching" were considered to have RP. They could have RP eighter on right or left hand or both.

*Carpal tunnel syndrome*

Paper I: Previous surgery for CTS right or left hand and/or symptoms and clinical signs of CTS (numbness or tingling in three radial fingers and positive Phalen and or Tinel's sign in the same hand) were considered to have CTS.

*Small nerve fibre neuropathy*

Paper II: Patients with impaired perception of cold, warmth, or pinprick test on second or fifth fingers. In the analyses we only included right hand.

*Large fibre neuropathy*

Paper II: Patients with impaired perception of touch or vibration, on second and fifth fingers. In the analyses we only included right hand.

## Severity grading of the neurosensory manifestations

The severity of the neurosensory injury was made according to the *International Consensus Criteria* (ICC) [71] (table 3b), with some modifications: in stage 3N 2PD was used instead of Purdue Pegboard. The evaluation was based on the hand with most pronounced symptoms or impairment.

- 0N: no numbness or tingling of fingers
- 1N: numbness or tingling of fingers
- 2N: as in stage 1N, and imparement in 2/3 sensory tests; perception of touch, temperature, or vibration
- 3N: as in stage 2N, and symptoms of impaired dexterity (a tendency to drop things or difficulty buttoning clothes) and reduced 2PD

*The severity was also graded according to the Stockholm Workshop Scale* (SWS) [232] (table 2b), with some modifications. The evaluation was based on the hand with most pronounced symptoms or impairment.

- 0SN: no numbness or tingling
- 1SN: implying numbness or tingling
- 2SN: as in stage 1SN and reduced perception of touch
- 3SN: as in stage 2SN and impaired 2PD.

## Severity grading of the vascular manifestations

The extent of finger blanching, could be scored by affected phalanges, also called Griffin scores [233]. The right and left hands were graded separately, and the result for the hand with the highest score (i.e., the highest number of blanching phalanges) was used. The ICC vascular grading is based on the Griffin score system [71] (Table 2a and Figure 22).

- 0V: No finger blanching episodes
- 1V: Griffin scores 1-4
- 2V: Griffin scores 5-12
- 3V: Griffin scores >12

Grading according to the SWS was possible in the field study since we had data on the frequency of finger blanching episodes, but this question was omitted for the patients. We did not look into each separate medical record, as above.

- V0: No finger blanching episodes
- V1: Occasional attacks, only distal phalanges
- V2: Occasional attacks, distal/middle phalanges on one or more fingers
- V3: Frequent attacks affecting all phalanges on most fingers

## Griffin scores

Blanching of the distal phalanx on the index, long, ring and little fingers corresponded to one point, middle phalanx: two, proximal phalanx: three points, and thumbs: four and five points for the distal and proximal phalanges (Figure 22). Scoring was done separately for the right and left hand. Among patients ten individuals reported episodes of finger blanching but had not filled in the hand-diagram. These were not included in the evaluation.

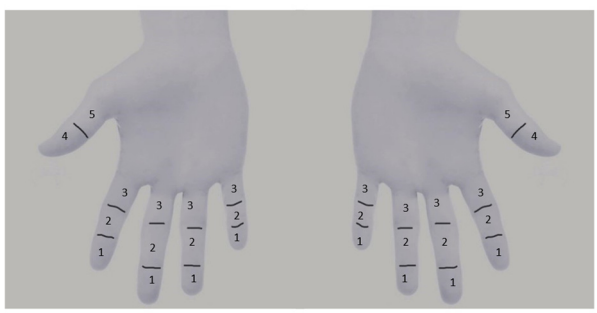


**Fig 22.** Scoring according to Griffin et al. [234].

## Exposure

*Paper I*

According to questionnaire for carpenters the most common work tasks with vibration exposure were framework, formwork, and demolition. Plastering was the most common work task, but with lower vibration exposure. A vast range of vibrating tools both with low and high frequencies and impact tools were used. The most common ones were screw drivers, impact drills and impact power wrenches, with interquartile ranges of vibration levels of 3–4, 8–15 and 5 – 9 $m/s^2$ respectively as measured according to the ISO 5349-1 standard. Painters usually did not use vibrating tools, however e.g., long reach sanders (vibration level interquartile range 3–4 $m/s^2$) could cause some exposure to vibration.

*Paper II*

All patients meet an occupational hygienist that assessed the historic and actual vibration exposure. Since there were no selection of the patient group, the group represents workers with different types of vibration exposure depending on work situation and job title, e.g., dentistry, demolition, and concrete work. The patients reported which tools they used, and for how long, in the present occupation. Past vibration exposure in other employments were also scrutinized. The vibration level for each tool was obtained from information by the manufacturer or from the vibration database at Umeå University, Sweden [Vibration Database (umu.se)], and calculated the A(8) value for the present job. "Vibration years" was calculated by multiplying number of years and A(8) values in each occupation, and these were summed for each patient. The use of impact tools was also assessed.

*Paper IV*

The daily vibration exposure [A(8); (m/s2)] was calculated from the tools' vibration magnitude and exposure time. Duration of vibration exposure in years was assessed.

## Biomarkers

Blood samples were collected after clinical examination, before noon, in 7 ml serum separation tubes with gel. After 30 minutes, serum was removed by centrifugation at 2000×*g* for 10 minutes, and the samples were stored at –80 °C until analyzed. Selected biomarkers were analyzed using commercially available immunoassays, eighter by enzyme-linked immunosorbent assays (ELISA) or multiplexed Luminex assays (Table 4). Samples with values below the limit of detection (LOD) were assigned a value of half the LOD in the statistical analyses.

**Table 4.** Biomarkers analyzed in serum

| Biomarkers | |
|---|---|
| 1. TM<br>2. vWf<br>3. ET-1<br>4. GFAP<br>5. HSP27<br>6. CGRP<br>7. MBP<br>8. TRPA-1<br>9. Caspase-3<br>10. Caspase-8<br>11. Galanin | ELISA kits |
| 12. ICAM-1<br>13. MCP-1<br>14. VEGF | Multiplexed immunoassays, (manufacturer 1)<br>Luminex platform |
| 15. MMP-1<br>16. MMP-12<br>17. b-FGF<br>18. β-NGF<br>19. HGF<br>20. NT-3 | Multiplexed immunoassays, (manufacturer 2)<br>Luminex platform |

# Statistics

## Paper I

*Logistic regression* was used to calculate odds ratios (OR: 95% CI) to compare the point prevalence of the different outcomes in carpenters and painters, reported as crude, age-adjusted and after sensitivity analyses including only participants without concurrent diseases (diabetes, cardiovascular-, rheumatologic-, thyroid disease and polyneuropathy and other peripheral nerve entrapments) values.

## Paper II

*Jonckheere-Terpstra trend test,* a non-parametric test, was used to assess if there was a rising trend in prevalence of symptoms, clinical findings, magnitude, and duration of vibration exposure, as well as the use of impact tools, with increasing severity on the SWS for neurosensory injury.

In the patient study, two different grading systems were compared using *Cohens kappa* and the *Svensson method*. In addition to the kappa value, that can be from 0 to 1, where 1 is perfect agreement and <0.1 is poor agreement, the Svensson method also gives information if there is a systematic shift towards lower or higher values. The systematic disagreement between scales can be expressed as relative position (RP), which indicates to which extent the distribution is systematically shifted towards higher or lower categories. The relative concentration (RC) shows whether the scores are concentrated towards the central categories of the scales compared to

the scores for the other scale. RP and RC values can vary from −1 to 1, where 0 means no difference and values between -0.1 – 0.1 are considered negligible. The statistical software also produced a Receiver Operating Characteristic (ROC) curve which enabled visual evaluation of systematic disagreement. Disagreement in position resulted in a concave or convex curve.

A *Venn diagram* was used to illustrate the overlap between large and small fibre affection as well as white fingers.

## Paper III

*Mann-Whitney U* tests, *a non-parametric test* was used to compare serum levels between groups, since serum levels of biomarkers were found not to be normally distributed.

*Sensitivity analyses* were performed where only individuals without variables that could interfere with the outcome were included. In the field study these were: diabetes, cardiovascular, thyroid, or rheumatic disease, impaired perception of vibration in the foot, or peripheral nerve entrapment in upper extremity/neck (except for carpal tunnel syndrome). In the patient study these were: gender, smoking, previous frostbite, and concurrent disease (cardiovascular disease, diabetes, thyroid disease, polyneuropathy or pharmacologically treated ADHD or migraine).

## Paper IV

*Jonckheere-Terpstra trend test* was used for assessing the association between biomarkers and increasing severity on the ICC for vascular and neurosensory injury.

*A generalized linear model* (GLM) was used to adjustments for both continuous and categorical data (age, sex, smoking, concurrent diseases, and previous frostbites). The crude associations, β with 95% confidence intervals, were presented. For each biomarker, associations between serum level and five potential confounders were tested: presence of concurrent diseases (yes/no), previous frostbites (yes/no), ongoing smoking, age (years), and sex (male/female), one at a time by the GLM. Confounders with $p<0.10$ were included in adjusted associations. Possible interactions (effect modifications) were tested by introducing an interaction term between the potential confounder and each of the four factors listed above (severity and exposure). When an interaction term with p-value $<0.05$ was found, the confounder was not included in the GLM model, instead data was dichotomized based on the effect modifier (for age <45 year and ≥45 years). On visual inspection of p-p plots of the residuals, the ones for CGRP were not normally distributed and CGRP was thus not included in the linear model.

# Summary of the results

## Paper I

- The criteria for neurosensory affection were fulfilled in 31% of the carpenters and 17% of the painters, age-adjusted OR 3.3 (CI 1.6–7.0).
- Neurosensory affection was found in 12 % of young carpenters (≤ 30 years old).
- The neurosensory manifestations dominated the clinical picture among carpenters; common and initiating symptoms were numbness or tingling (26%) and cold intolerance (24%) in fingers.
- The vascular manifestation, Raynaud´s phenomenon (RP), was not so prevalent, 9% among carpenters and only 2% among young carpenters. Painters showed about the same prevalence of RP (8%). More severe stages of RP were only fulfilled by few carpenters.
- The prevalence of carpal tunnel syndrome was higher than in the general population in men for both carpenters (10%) and painters (11%).

## Paper II

- There was poor agreement between the SWS and the ICC scales for grading the severity of the neurosensory component of injury. Cohens kappa was 0.22 and the percentage of agreement with the Svensson method was 40%.
- There was a systematic shift so that 60% scored less with the ICC than with SWS. The ROC curve showed a concave shape, with lower grades of severity with the ICC than with SWS.
- The most prevalent symptoms among patients were numbness (91%), and cold intolerance (86%).
- Small nerve fibre neuropathy overlapped with large fibre neuropathy and RP. It was more prevalent though to have small fibre than large fibre neuropathy. To only have large fibre neuropathy or only RP was very rare.
- Prevalence of symptom, clinical findings and exposure were associated with increased severity grading on SWS SN.
- Eight patients did not have the symptom numbness and were hence graded with “0” for neurosensory manifestations.

## Paper III

- Patients with hand-arm vibration injury showed elevated serum levels of ICAM-1, MCP-1, TM and HSP27 compared to controls.
- Patients with RP showed elevated serum levels of TM, vWF, CGRP, and caspase-3 compared to patients without RP.
- Patients with only the neurosensory component of injury (without RP), showed elevated serum levels of ICAM-1, MCP-1 and HSP27 compared to controls without RP.
- Serum levels of VEGF, b-FGF, HGF, MMP-1, caspase-8, GFAP, MBP, Et-1, TRPA1 and galanin showed no differences among the studied groups. Serum levels of MMP-12, β-NGF and NT-3 were below the detection limits in all samples.

## Paper IV

- Serum levels of TM, vWf, CGRP, HSP27, and caspase-3 were all positively associated with severity grading of RP.
- In a linear model, serum levels of TM, vWf, and caspase-3 showed linear associations with increasing severity of RP. For HSP27 there was an association but not linear.
- Serum levels of CGRP were positively associated with the severity of neurosensory manifestations.
- CGRP could not be tested in a linear model.
- There were no linear associations between biomarkers and sensibility index for vibration perception thresholds (VPT), nor for current magnitude of exposure as (A8)-value, or years of exposure.
- For ICAM-1 and MCP-1, there was no association with severity of neither the vascular, nor the neurosensory components of injury.

# Discussion

## The clinical picture

*Neurosensory and vascular manifestations*

The neurosensory component of injury dominated among both carpenters and patients (Table 5), which is in line with previous studies [4, 235, 236]. A risk nomogram calculates the neurosensory component to precede the vascular one by a factor three [106]. The prevalence of RP among patients was 49%. In the field study the prevalence was much less (9%), but in line with a nationwide study in UK, where 14% of carpenters reported white fingers [237], and with a Swedish cross sectional study, where 13% of construction workers reported white fingers [238], but yet higher than among men in the general population [31, 40].

*Carpal tunnel syndrome*

Both carpenters and painters had a higher prevalence of carpal tunnel syndrome (10 % and 11% respectively) than that reported among men in the general population (2%) [81]. This might be due to vibration exposure in combination with high ergonomic workload with hand intensive work tasks. Both hand-intensive work and vibration exposure are risk factors for carpal tunnel syndrome [79, 239]. A prevalence of 7-35% has been reported among vibration-exposed workers in various epidemiological studies [4, 60, 80], with an elevated POR of 3.4 (1.4 – 8.3) among stoneworkers, compared to controls [240, 241]. In a systematic review and meta-analysis showed an effect size of OR 2.9 comparing workers exposed to vibration from hand-held tools with a non-exposed group [4].

**Table 5** Descriptives, symptoms, clinical findings and exposure in study participants.

| | Field study | | Case series study | |
|---|---|---|---|---|
| | Carpenters n=193 (%) | Painters n= 72 (%) | Patients n=92 (%) | Controls n=51 (%) |
| Age (median range) | 40 (17 – 65) | 46 (23 – 68) | 45 (21-64) | 42 (26 – 62) |
| Female | 0 | 0 | 7 | 18 |
| Current smokers | 16 | 22 | 15 | 4 |
| Current nicotine user | 45 | 44 | 44 | 35 |
| Cardiovascular disease | 8 | 11 | 20 | 14 |
| Diabetes | 3 | 1 | 7 | 4 |
| Thyroid disease | 1 | 1 | 5 | 2 |
| Rheumatic disease | 2 | 3 | 0 | 0 |
| Numbness | 26 | 22 | 92 | 14 |
| Cold intolerance | 24 | 8 | 86 | 12 |
| Impaired fine motor skills | 7 | 3 | 67 | 8 |
| Impaired grip strength | 18 | 13 | 71 | 8 |
| Raynauds´s phenomenon | 9 | 8 | 49 | 10 |
| Touch (Monofilament) | 31 | 35 | 45 | 12 |
| Vibration perception (VPT) | 22[a] | 14[a] | 41 | - |
| Cold (RollTemp) | 6 | 8 | 77 | - |
| Warmth (RollTemp) | 4 | 4 | 51 | - |
| 2PD | 9 | 3 | 44 | - |
| Seniority | 20 (0-50) | 24 (3-54) | 20 (2.6 – 46) | - |
| Current exposure [A(8); (m/s2)] | estimated >2.5>5.0 | if grinders used<2.5 | 2.8 (0-7.0) | - |

[a] VPT 125 and 250 Hz only

## Small and large fibre neuropathy

Among patients the clinical findings were dominated by small fiber neuropathy with impaired discrimination of cold or warmth. Small-fibers were most prevalent (83%), followed by affected large-fibers (54%), i.e., an impairment measured with VPT and or Monofilament. Isolated affected small-fibers exclusively were ten times more common than only affected large-fibers. Only 2 individuals had only large fiber neuropathy. These finding are supported by previous studies; a longitudinal study, comparing vibration exposed workers with controls, showed that the perception of temperature (small nerve fibers) was more affected than perception of vibration (large nerve fibers) [115]. An associations between thermal sensory impairment and cumulative exposure to vibration was shown [242], yet in a longitudinal study on vibration-exposed workers, a low cumulative vibration dose did not significantly affect thermal perception thresholds, whereas age did [243]. Impaired discrimination of cold was the most prevalent clinical finding, followed by impaired discrimination of warmth (77% and 51% respectively). The threshold for the perception of cold has been suggested as the best indicator of early neurosensory impairment since it showed greater sensitivity and specificity than the detection of warmth in fingers with numbness or tingling [244]. Early changes in carpal tunnel syndrome have suggested to involve small nerve fibers with impaired thermal

sensation, before larger myelinated nerve fibers get affected. Cold detection thresholds (Aδ-fibers) were significantly correlated with the severity of CTS symptoms, whereas no significant correlation was found between warmth perception thresholds (C-fibers) and vibration perception thresholds (Aβ-fiber), suggesting that damage to Aδ-fiber plays a major role in the pathogenesis of CTS symptoms [245]. In contrast there are other studies implying that large, myelinated fibers are more vulnerable to stress, than unmyelinated nerve fibers and degenerate early during compression [60]. In an animal study a destruction due to vibration exposure occurred earlier and more frequent to the myelin sheath than injury to the axon [201]. Axonal degeneration with nerve fibre loss is considered as a late and severe stage of compression nerve injury [217]. In an animal model, the reduction in nerve conduction velocity correlated with the increased in disrupted myelinated axons. After one week of vibration exposure there was a recovery, but following two weeks of exposure the recovery was absent, implying a permanent injury [200].

## Cold intolerance

In both the field study and among patients' cold intolerance was a very prevalent symptom. Cold intolerance in vibration exposed individuals needs early preventive strategies since the condition entails a poor prognosis and negative impact on quality of life [46, 118]. Almost one fourth of carpenters claimed to have cold intolerance, which is in line with another study of vibration exposed workers [246]. Compared to painters the odds ratio was threefold. The prevalence in painters was 8% which was the shown in the general population in northern Sweden (5 -15%) [30]. Among diagnosed patients the prevalence was as high as 86%. In the biomarker study we had the ambition to look specifically at biomarkers in relation to cold intolerance. However, since 44 of 45 patients with RP also had cold intolerance, it was not possible to look at cold intolerance separated from RP since the remaining groups were too small. Hence elevated levels of some biomarkers shown in patients with RP compared to patients without RP also reflect that they have cold intolerance. The exact site of injury and the pathophysiological mechanisms behind warrants further unravelling in a study specifically addressing cold intolerance. In figure 23(a-c), serum levels of CGRP, caspase-3 and HSP27 in patients without RP; but with or without cold intolerance. We choose not to present this data in paper III since groups are too small for statistical analyses. It would have been interesting to look separately on the association between these biomarkers and the severity of cold intolerance, using e.g., the cold intolerance questionnaire (CISS) [53].

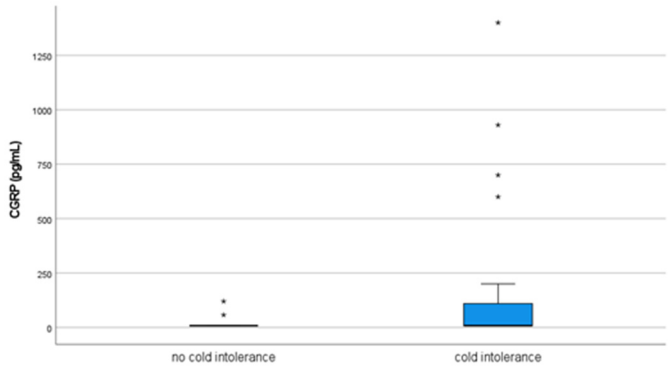
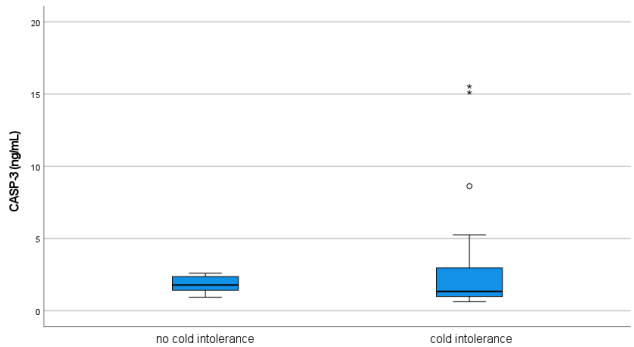
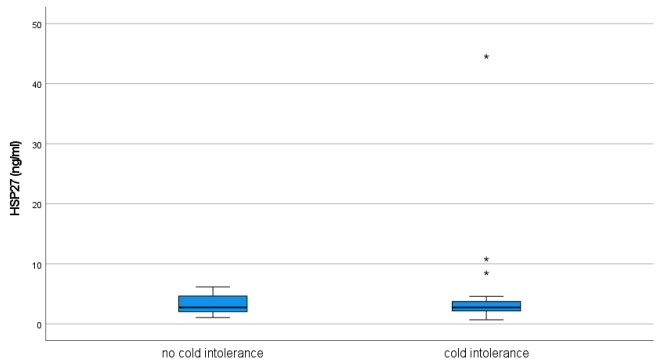



**Figure 23 a.** Serum levels of CGRP in patients with no cold intolerance (n=11) and patients with cold intolerance (n=36), among patients withouth RP (n=47).




**Fg 23 b.** Serum levels of caspase-3 in patients with no cold intolerance (n=11) and patients with cold intolerance (n=36), among patients withouth RP (n=47).




**Fg 23 c.** Serum levels ofHSP27 in patients with no cold intolerance (n=11) and patients with cold intolerance (n=36), among patients withouth RP (n=47).

# Grading of injury – symptoms and clinical findings

## Need for un update?

In the carpenter study we defined a new clinical condition called neurosensory affection, which meant having one symptom from the questionnaire and one impairment on clinical examination with the neurosensory tests. One third of the carpenters fulfilled our criteria for neurosensory affection, which is of the same magnitude as neurosensory disturbances reported among forestry workers (33%) [247]. However, these neurosensory manifestations are not captured by SWS, nor the ICC scales. 17% of carpenters scored with SWS and 6% with ICC neurosensory grades 2 or higher. Yet, half of the carpenters with neurosensory affection reported symptoms daily, and one fifth that they interfered with daily life activities, indicating that these manifestations are not at all negligible.

In the patient study there were 8 patients not included in the neurosensory scales SWS and ICC since they did not have the symptom "numbness or tingling", which is the inclusion criteria for the first grade. Patients graded as "SWS SN0" exhibited symptoms of cold intolerance, impaired manual dexterity, and impaired grip strength. As mentioned earlier, cold intolerance is an important symptom to address and has shown a high agreement with Stockholm workshop scales [64]. However cold intolerance was not addressed in the original SWS grading and was omitted in the final ICC grading system, although it was commented on in the discussion [71]. There are conflicting results whether the symptom should be regarded as belonging to a severity grading of vascular or neurosensory origin. In a prospective study, at group level, the risk of developing RP significantly increased among those with a previous sensation of cold hands [248]. There are suggestions of an inclusion in the vascular grading, as an early sign of RP as "grade 0.5"[249]. However this was disagreed by others [43] since redundant evidence points towards a neurosensory origin, although the pathophysiological mechanisms behind cold intolerance are not fully elucidated. Studies underpins an association between cold intolerance and small fibre neuropathy since poorer performance in thermal perception thresholds were obtained [250], however another study concluded that QST offered little aid in diagnosing cold sensitivity [251]. Furthermore, in grade "SWS SN0" 8 patients showed clinical impairment indicating small fiber neuropathy, where impaired discrimination of cold (57%) was more frequent than impaired perception of warmth (43%). This indicates that both the SWS and ICC are not capturing important neurosensory manifestations in these patients compatible with hand-arm vibration injury.

When comparing the two scales SWS and ICC, it was shown that 60% of the patients received a lower grade of severity by ICC. The reason for this is that ICC is more stringent. To obtain the criteria for grade 2N sensory perception in two out of three

sensory modalities have to be fulfilled, whereas the SWS requires impairment in only one sensory test. For grade 3N testing with Purdue Pegboard is required [71] while in SWS SN3 2PD can be used. The clinical use of 2PD screening to detect early injury in HAVS has been questioned, since impaired 2PD is claimed to appear late in neurosensory injury and both nerve fiber density and the neurosensory input to the sensory cortex in the brain seem to be involved. Interestingly among the patients, impaired 2PD was more prevalent than impaired manipulative dexterity measured with Purdue Pegboard, indicating that 2PD still might have a place in the scale. Since grade 2 is a watershed for taking action to hinder further disease progression, the interpretation of the grades if changing to ICC should be updated.

# Pathophysiological mechanisms and biomarkers

The selection of 20 biomarkers was based on what we found most relevant, but also what was practical and possible to analyse in serum in terms of half-lives, diurnal variations, and specificity. Among the patients, the prevalence of RP increased with rising severity of the neurosensory component. There was a great overlap between RP, small-, and large fibre neuropathy, thus, whether the biomarkers that were elevated among the patients reflect an injury related to the vascular and/or the neurosensory component of injury is difficult to decipher. Below is an attempt to structure the biomarkers into different plausible pathophysiological explanations, however many of the biomarker can fit into more than one category and the categories can be overlapping. Although biomarkers were related to severity, levels of biomarkers cannot be interpreted in terms of severity since we don't have information about disease progress. This need to be further investigated.

## Vaso-protection and cold intolerance

In the biomarker study patients with RP showed elevated levels of CGRP compared to those without RP. In addition, there was also an association between serum levels of CGRP with increasing severity on the neurosensory scale (ICC 0-3N, but not for the vascular scale (ICC 0-3V). CGRP, a very potent vasodilator is stored in nerve terminals of C-fibres and Aδ-fibres [180]. Vasoconstriction has been observed as a direct effect of vibration exposure. An explanation for the increased levels of CGRP could be that CGRP is released from small Aδ and C-fibres in response to injury or imbalance. Increased levels of CGRP leads to neurogenic vasodilation, attraction of inflammatory cells and has a protective function. There was an inhibit  in intima hyperplasia and expression of the inflammatory marker MCP-1 on CGRP administration in a condition with induced inflammation [180].

If vibration exposure continues this can eventually lead to loss of CGRP releasing neurons and instead lower levels of CGRP. A reduction of CGRP-staining nerves in histopathological examination of biopsies from patients with primary RP, secondary RP due to scleroderma, and in patients with vibration-induced RP has been shown [183-185].

Furthermore, CGRP has been associated thermo-sensing channels, TRPA-1 that has been associated with cold intolerance [194, 252], and TRPV1 that has been associated with inflammatory neurogenic pain [56, 193, 196, 253] (Figure 24). A possible link with the prevalent symptom cold intolerance in patients with hand-arm vibration injury is therefore highly interesting and warrants further investigation. In an animal model cold hypersensitivity could be induced in mice by subcutaneous administration of a TRPA-1 agonist, while TRPA-1 antagonist reduced cold hypersensitivity [194]. TRP might be interesting candidates for pharmacological therapies to further explore for vibration induced injuries. Blocking TRPA1 have been suggested as a therapeutic target for treating cold hyperalgesia caused by inflammation and nerve injury [197]. Recover in sensory impairement was shown on capsaicin-induced upregulation of CGRP in the dorsal root ganglion neurons via TRPV1 receptor [253]. If the clinical manifestations of cold intolerance and the clinical presentation of impaired thermal sensation to cold has an association with the thermoreceptors, nerve fibers, or supplying vasculature, as a structural injury or functional loss, warrants further investigation.

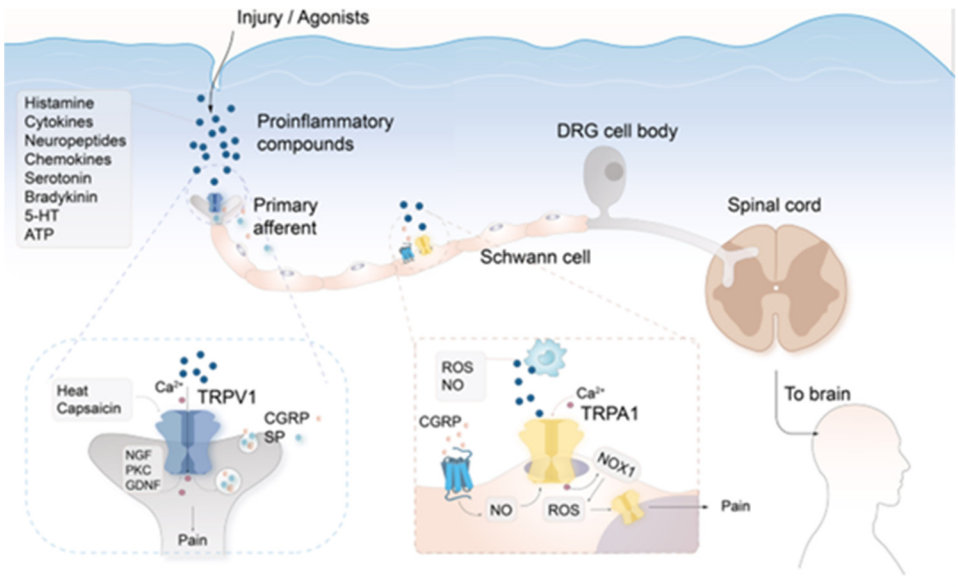



**Figure 24.** Illustrating the association between TRPA1, TRPV1, CGRP and the somatosensoric input of pain. Figure reproduced con permission from CC BY 4.0 Copyright © 2023, Zhang et a. [57].

## Shear stress, inflammation, and vascular remodelling

The elevated levels of **ICAM-1** in patients compared with controls are in line with a previous study [19]. Upregulation of ICAM-1 has been shown in response to both low and high amplitude of cyclic strain applied [254, 255]. Patients compared to controls also showed elevated serum levels of **MCP-1** compared to controls. MCP-1 has been associated with an inflammatory response [156, 157]. Thus, the elevated levels of ICAM-1 and MCP-1 suggests an inflammatory response in vibration injury as a response to shear stress, cyclic strech, possibly due to injury or dysfunction of the endothelial cells or other structures.

Furthermore, patients with RP hade elevated serum levels of **vWf** compared to patients without RP and there was an association between the biomarker and increased severity of RP according to ICC V severity staging. The association remained in a linear model, adjusting for confounder. Increased levels of vWf have been associated with inflammation and intimal hyperplasia [256]. In conditions with high shear stress, it has been shown that globular-shaped vWF unfolds into long-chain structure, making the adherence of platelets to the injured endothelial surface easy and enhancing further leukocyte recruitment [256]. Smooth muscle cell proliferation seem to occur also under levels of low shear stress with intact endothelium and without platelet activation [256]. In vitro studies have shown a dose-response relationship between intimal hyperplasia and the expression of vWF [257]. These findings underline the results in animal studies, where structural changes in blood vessels with increased smooth muscle thickness have been shown in vibration injured individuals and in vibration exposed rats [15, 18, 96]. Hypothetically, increased levels of vWf could be caused by vibration-induced changes in shear stress and cyclic stretch to the vessel wall [258, 259]. Furthermore, elevated levels of vWf have been shown in patients with systemic sclerosis, indicating an inflammatory response and vasculopathy [171]. Levels could be related to disease severity. Also, levels were elevated in individuals with RP, that later on developed a systemic connective tissue disease [124]. Thus, elevated levels of vWf in patients with vibration induced RP as in our study, might reflect an ongoing mechanism with endothelial dysfunction, inflammation, and intimal hyperplasia.

## Endothelial injury or dysfunction

Our study confirms the results of other studies showing elevated blood levels of **TM** in vibration injured individuals compared to controls [19, 170, 189]. For subgroups of patients TM was elevated in patients with RR compared to patients without RP, while for patients with only the neurosensory component of injury (without RP) there was no elevation in TM compared to controls. These findings, together with the results that TM was associated with the severity of RP, is in line with the idea that TM is associated with endothelial injury or dysfunction. TM is an endothelial

surface protein, normally expressed at low levels in serum, thus, this elevation might reflect a pathological condition in the endothelial cells due to vibration exposure. Elevated blood levels of TM have been associated with endothelial injury, e.g., in cardiovascular -, inflammatory-, infectious -, and metabolic diseases [169]. Interestingly the elevated trend for TM in patients with RP showed a decline in in the most severe stage. One hypothesis could be that more severe vascular manifestations reflect a more pronounced injury or dysfunction where the endothelial cells lose their ability to produce TM.

## Neuroprotection

Serum levels of **caspase-3** were elevated in patients compared to controls and in patients with RP compared to patients without RP. In addition, elevated serum levels were positively associated with the severity of RP, also in a linear model, after adjusting for concurrent diseases. Increased levels of caspase-3 have been measured as an apoptotic response in Schwann cells, to balance the proliferative response after a nerve injury [21].

Serum levels of **HSP27** were not shown to be elevated in patients with RP compared to patients without RP. However, serum levels of HSP27 were positively associated with the severity of RP, which remained after adjusting for confounding, e.g., concurrent diseases, however the association was not found to be linear. This is an interesting result, and a plausible explanation could be that RP reflects nerve fibre injury. An idea further supported by the results that patient compared to controls, as well as patients with only the neurosensory component of injury compared to controls, showed elevated levels of HSP27. Although as shown CGRP (with a plausible association with small nerve fibre neuropathy and cold intolerance), HSP27 did not seem to be linked to the expression of cold intolerance (reservation for few individuals in the groups). What exact mechanisms and structural changes that possibly is behind the elevated levels HSP27 needs to be further explored. HSP27 has shown to protect nerve structures under stress [225, 228] and is associated with compensatory neuroprotective mechanisms to preserve function, as seen in subjects with diabetes neuropathy [202, 226, 230]. The elevated levels of HSP27 could therefore indicate the same mechanisms, since similar structural changes in nerve biopsies from the posterior interosseous nerve, i.e., nerve fibre degeneration, demyelination and fibrosis, as seen in subjects with diabetes neuropathy have been shown in vibration injured patients [16]. A previous register-based study did not show elevated levels in hand-arm vibration exposed workers compared to individuals without such exposure [224], however there was no information about injuries from hand-arm vibration, nor the timing of exposure.

## Progression of injury

Hypothetically, injury can start in the small nerve fibres innervating blood vessels (nervi vasori) or in the small blood vessels supplying nerves (vasa nervorum) [55], a pathophysiological mechanism described in diabetes neuropathy [260]. Structural changes in both blood vessels and nerves have been observed in relation to vibration exposure e.g., disrupted endothelial cell lining [18] and loss of CGRP-containing nerve fibres [184]. Peripheral nerves are supplied by a system of longitudinal blood vessels known as the vasa nervorum. The reciprocal signalling between nerves and blood vessels, may serve to guide and support under development, as in regeneration after injury. Studies in embryonic mouse limb skin shows that arteries, are aligned with peripheral nerves. Mutations in peripheral sensory nerves or Schwann cells, affects the angiogenesis and axon get misrouted, suggesting that peripheral nerves provide a template that determines the pattern of blood vessel branching and arterial differentiation in the skin, via local secretion of various substances, e.g., VEGF [261]. As for TM, several of the biomarker levels did not seem to be more elevated in ICC 3V compared to ICC 2V. A mechanisms where dysfunctional or injured endothelial cells first leads to unregulated release, measured as an increase of substances or compensatory mechanisms, but eventually after repeated trauma, the endothelial cells or neuropeptidergic release from nerve fibres is exhausted due to depletion is plausible [180, 183-185].

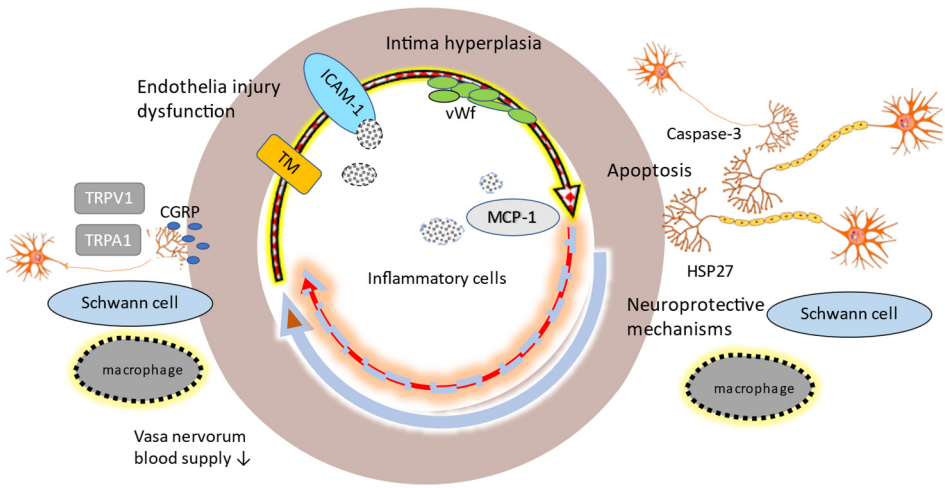



**Figure 25.** Schematic illustration of plausible pathophysiolological mechanisms: Shear stress and mechanical forces on the vessel wall increase vasoconstriction ↔ hypoperfusion of nerve fibres ↔ dysfunctional/ injured endothelial cells (increase in TM) ↔ further polarizes towards vasoconstriction ↔ compensatory mechanisms; CGRP induces neurogenic vasodilation ↔ adhesion molecules, ICAM-1 and MCP-1, enhances the migration of inflammatory cells ↔ vWf elongates and adheres to the endothelial cells, starting a process of intima hyperplasia with further narrowing of the vessel lumen ↔ caspase-3 and HSP27 try to restore the balance by apoptosis and neuroprotective mechanisms. Hypersensitized small nerve fibres via NO-ROS↔ CGRP ↔ TRPV1A and TRPV1 induce cold intolerance and inflammatory neuropatic pain.

## Other biomarkers

For clinical implication a biomarker must be measurable and linked to pathophysiological explanation [262]. The biomarker candidates included in the current study were selected to reflect endothelial injury or dysfunction, inflammation, nerve injury and tissue remodelling of extracellular matrix. For some biomarkers there are epidemiological and experimental studies specifically associated to vibration exposure, other biomarkers are included since they have been studied in relation to Raynaud's phenomenon and nerve injury repair. In this group of patients, we could not detect a difference for some of the biomarkers analysed. This could reflect many other factors. To be able to detect a true difference the sampling must be performed in the adequate time interval of the disease, the right sample matrix, the assey must provide a sufficiently low detection limit, and the biomarker should be stable in storage over time. Elevated levels of GFAP and neurotropic markers could not be shown, nor biomarkers associated with angiogenesis, remodelling, and fibrosis, e.g., MMP-1, MMP-12, HGF, basic-FGF, VEGF [131]. There are many other interesting candidates of biomarkers to study, that we in this study for practical reasons did not include, e.g., nitric oxide (NO), other thermoreceptor, e.g., TRPV1. Furthermore, biomarkers for bone metabolism are interesting. The effect of mechanically induced whole-body vibration on bone metabolism has been studied [263, 264].

# Exposure

## Association with exposure

*Paper II*

Among patients a statistically significant trend was seen between exposure and SWS grade. Longer exposure to vibrating tools, expressed as duration in years and in terms of vibration years, and a higher prevalence of work with impact tools were related to higher grades. In fact, 70% of the patients classified as grade SWS 3SN had worked with impact tools.

*Paper IV*

There were no association between serum biomarkers and the current exposure magnitude nor the duration of exposure. However, the interpretation of this is complex, since we did not take into account the underestimation of the true exposure due to the frequency weighting e.g., the use of impact tools. An increased risk for cold intolerance (OR 2.8, 95% CI 1.7-4.6) was reported in male workers using impact tools [51].

## An ongoing risk

In *paper I* one out of ten young carpenters had signs of neurosensory injury. This indicates an ongoing risk from vibration exposure. The prevalence of RP was however low, only 2% compared to 13% among carpenter 30 years or older (Table 6). This is in line with a systematic meta-analysis showing that on equal exposures, neurosensory injury occurs with a 3-time factor shorter latency than Raynaud's phenomenon [106]. Thus, we can presume that adverse health manifestations among young carpenters were signs of early vibration injury, it is therefore important to notice the high prevalence of cold intolerance (28%) in this group. In *paper IV* more than half of patients had an ongoing vibration exposure above the action limit value, median 2.8 $m/s^2$ (range 0.0-7.0).

**Table 6.** Prevalence of symptoms, findings and clinical conditions in carpenters younger or older than 30 years.

| | Carpenters ≤30 years n=60 (%) | Carpenters >30 years n=133 (%) |
|---|---|---|
| *Symtoms from questionnaire:* | | |
| Finger blanching (RP) | 1 (2) | 17 (13) |
| Increased sensation of cold | 17 (28) | 50 (38) |
| Pain in fingers when cold (cold intolerance) | **15 (25)** | **32 (24)** |
| Numbness/tingling | 10 (17) | 41 (31) |
| Impaired perception of touch | 5 (8) | 23 (17) |
| Impaired perception of cold | 3 (5) | 16 (12) |
| Impaired perception of warmth | 3 (5) | 20 (15) |
| Impaired manual dexterity | 7 (12) | 20 (15) |
| Neck-shoulder problems | 18 (30) | 49 (37) |
| | | |
| *Impaired clinical findings with:* | | |
| VPT | 12 (20) | 30 (23) |
| Monofilament | 5 (8) | 55 (41) |
| Temprolls cold | 2 (3) | 10 (8) |
| Temproll warm | 2 (3) | 5 (4) |
| 2PD | 3 (5) | 14 (11) |
| *Clinical conditions* | | |
| Neurosensory affection | 7 (12) | 50 (38) |
| Carpaltunnel syndrom | 2 (3) | 18 (14) |

# Methodological considerations

Strengths in this work is the large number of study participants clinically examined by skilled personnel, with validated test methods and according to standardized protocols. There are also some limitations that should be addressed.

## Validity and reliability

Since the carpenter study is a cross-sectional study and there is a possibility of *selection bias* due to a healthy worker effect, i.e., workers with occupational disease may have left the occupation. Normally, such an effect will decrease the effect size. Furthermore, the examined group of carpenters belonged to well-functioning big and medium sized, companies and the workers we examined were probably not the most exposed group, since the work tasks with high concern vibration exposure, e.g., demolition are laid out to hired personnel, often temporarily workers out of reach for the health surveillance program. In addition, exposure estimations from questionnaire showed that carpenters exceeded the exposure action value in most work tasks and sometimes also, the action limit value, while painters exceeded the limit value only occasionally from oscillating grinders [265]. Also, the lower participation rate among painters (67%) could mean that painters with problems in their hand tended to come to the examination. There could also be some information bias, since it is possible that carpenters may be more aware of symptoms in their hands in relation to vibration exposure than painters. Further, in the postal survey, participation rate was less than 50%. Since carpenters in the postal survey reported symptoms with a higher prevalence than the investigated carpenters, it could mean that the once responding to the survey were the ones with hand problems. Even, if those 50 % percent that did not answer had problems, e.g., the true prevalence would be 23% instead of 46%, and thus be in line with the group of examined carpenters (24%). Hence, we believe the examined group of carpenters is representative for carpenters in general in a Swedish work setting Taken together, we believe that all these possible biases would rather result in lower differences in OR between the groups and tend to underestimate the real health situation.

In the patient study, the group of hand-arm vibration-injured patients we believe that these patients are representative for such patients. All consecutively referred patients for investigation were invited to participate and only eight declined. We have a long experience from examining such patients and the ones included do not

differ from the typical patients in any systematic way. In the control group we asked workers that were heavy smokers not to participate, as blood samples were also taken for chromium, for a separate study. The prevalence of smokers was lower in that group than among the patients, and we therefore performed sensitivity analyses including only non-smokers. In all papers there is a possibility of recall bias when asking about e.g., symptoms, injuries, or vibration exposure in the past. However, this would be the same between the compared groups.

*Case definitions*

Episode of finger blanching were assessed based on medical history and filling in a hand-diagram showing the worst episode of finger blanching. Self-reporting of RP has shown a predictive value of 80% when followed up by medical interview [266]. A strength is that RP was graded according to guidelines in the ICC V and established on patients' history of number of phalanges affected on "the worst" episode of finger blanching. This gives an estimate of severity that does not depend on climate or whether the patient works indoors or outdoors, which makes the estimate more reliable. The case definition of RP was to have episodes of finger blanching on eighter hand. The case definition of neurosensory component was however based on symptoms and clinical finding in only the right hand. Among patients 9% were left-handed. Since handling vibrating work tools involves both hands) we believe that by only including the right hand in the case definitions of the neurosensory component in paper I and II would not have biased the results to a major extent. However, almost one fourth of carpenters and only of 8% of painters reported other injuries to the hands. Repeating the analyses for neurosensory affection, only including individuals without "injury relevant to hands", the OR remained the same in all models.

*Clinical examination*

Due to practical limitations, the discrimination of warmth and cold was assessed by RollTemp® performed by a physician and based on verbal instructions without an explicit written protocol and with no temperature check before. Since some patients´ hands could have become cold, this could have resulted in a false positive result concerning the discrimination of cold. The conduction velocity is slower in the thin unmyelinated C-fibres than in the myelinated Aδ fibres, which could have affected our results, leading to under-reporting of the true impairment of warmth perception. However, we do not suspect that there was any systematic bias in these examinations between patients with different severity of vibration injury. Yet, it would have strengthened the precision performing the test with a standardized and quantitative method e.g., temperature perception thresholds with esthesiometry, rather than only measure qualitatively with RollTemp®. In the field study we had to adjust the time a clinical examination could take. Hence a shortened version of the standard clinical examination with VPT, only including two frequencies was performed. The ICC for grading the neural component of injury suggests including at least the two

frequencies; 32 Hz and 125 Hz. The ICC suggests 32.5 and 125 Hz. In a study aimed to present the somatosensory profile with QST, it was shown that VPT was the test method that showed the greatest impairment in vibration injured individuals compared to controls. [60]. Another study showed more impairment at 150 Hz compared to 20 Hz in a group of vibration injured patients [231]. In our study it was more prevalent to have an impairment in 250 Hz than 125 Hz. For 250 Hz the prevalence of impairment was almost the double in carpenters compared to painters, suggesting that also higher frequencies above 125 Hz should be tested for. Thus in line with a study on roadworkers, where the greatest threshold elevations were identified at 250 and 500 Hz [267], we suggest that also frequencies above 125 are important to include in the clinical examination to detect early injury.

Also, the perception of touch with was only tested the perception of touch with monofilaments once on each tested finger, although it is recommended that this should be done three times if no response is elicited. This could have influenced the precision of data. Since epidermal thickness can influence perception of touch, the cut off value of 3.61 instead of 2.83 (normal perception of touch) for Semmes-Weinsteins monofilament was used to reduce the risk of false positive results. Since no significant difference could be detected for impaired perception of touch between carpenters and painters, it is possible that we might have underestimated the OR, by increasing the cut off value. Comparing severity grading of neurosensory manifestations with only self-reported symptoms showed a 60% concordance with severity grading based on quantitative sensory tests with VPT and Purdue Pegboard in individuals exposed to hand-arm vibration [72]. Taken together, we consider clinical the conditions of the neurosensory component reliable since they are based on a combination of both symptoms and clinical findings.

*Biomarkers*

The biomarker candidates included were selected to reflect endothelial injury or dysfunction, inflammation, nerve injury and tissue remodelling of extracellular matrix. For some biomarkers there is epidemiological and experimental studies specifically associated to vibration exposure, other biomarkers are included since they have been studied in relation to Raynaud's phenomenon and nerve injury repair. A large number of biomarkers were tested which increases risk of mass significance effects, and a type I error, i.e., incorrectly rejecting a true null hypothesis. Still, we choose not to perform Bonferroni correction, since the included biomarkers were not chosen randomly, and we were interested in the specific outcome and pathophysiological mechanisms for each individual biomarker. However, if the biomarkers were to be implemented for practical use to determine grade of injury, the correction of significance levels should be addressed.

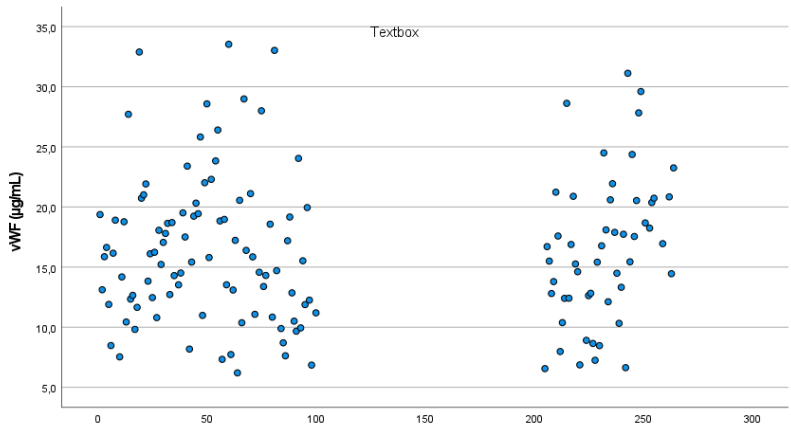
To be able to detect a true difference the sampling must be performed in the right time interval of the disease, the right sample matrix, and stability in storage over time. To be used clinically a biomarker should be measurable and be linked to a

pathophysiological explanation [262]. Differences in blood biomarker concentrations due to diurnal variations were minimized by collecting all blood samples before noon. Repeated measures of serum biomarkers would have strengthened the reliability of the results but were not possible due to practical and economic reasons.

For associations between biomarkers and severity of neural injury, VPT was chosen since the continuous data was best suited for the linear regression model. VPT has been shown to be the best indicator of neural injury among vibration injured patients, however since neuropathy is often diffuse and with overlapping signs of small and large fibre neuropathy, no single best method is recommended, but rather a combination of different tests [60]. Since VPT only reflects impairment in the larger Aβ fibres, a more pronounced impairment in only small fibres could have been missed out. About 20% of patients had only small fibre neuropathy.

For some biomarkers the concentration in serum and plasma respectively may differ depending on the clotting process. VWf is involved in the coagulation process and can be measured in both serum and plasma. A study showed slightly lower levels of vWf in serum compared to plasma, but the difference was not significant in a patient group [176]. Thus, we believe that the serum samples were reliable.

In addition, the recruitment of the control group was delayed due to pandemic of Covid 19. There is a maximum of four years between the first and last blood sample storage. The difference in storage time before analysis could add to the uncertainty of the results. The concentrations of biomarkers may potentially be biased towards higher biomarker concentrations in samples from controls, depending on the stability of the biomarkers, due to a shorter storage time, however, there no temporal trend was observed for any of the biomarkers (Figure 26).




a

**Figure 26.** Serum levels of vWf (y-axis) towards Patients (left) and Controls (right) on the x-axis.

There was no association between serum levels of any of the biomarkers and vibration exposure. We had not taken into account the use of impact tools that could have biased the results. Impact tools have shown nerve and vessel injuries in experimental studies on rat tail [96, 98] and increased response in an cold provocation tests in individuals with vibration induced RP [93].

## Confounding

In the field study painters was chosen as a control group, as they belong to the same socioeconomic strata and as carpenters have hand-intense work, often above shoulder level. In fact, painters reported higher a prevalence of problems with pain from hands-elbows, neck, and shoulder than carpenters, indicating a high ergonomic workload on upper extremities. Another possible confounder could be that painters had polyneuropathy from historic use of solvents. These confounders have been addressed in the sensitivity analyses, by only including individuals without possible nerve entrapment and only individuals without signs of generalized neuropathy.

In all studies the prevalence of *age, gender, nicotine use, and concurrent diseases* differed between the studied groups, thus, sensitivity analyses were performed. For VPT, Purdue Pegboard, and grip strength with Jamar dynamometer, but not for RollTemp® instrument, Monofilaments and 2PD there are standardized reference values adjusted for age. Impaired performance with thermal perception thresholds, have been shown with increasing age [268]. For Purdue Pegboard we used different cut off values for age <45 and >45).

Nicotine in both cigarette smoke and snuff is vasoconstricting. Increased reactivity in a cold provocation test was shown in tobacco users with vibration induced RP with [269]. The instruction to all participants was to not use nicotine in any form one hour prior the examination. In addition, cigarette smoking injures blood vessels by oxidative stress. Trombangitis obliterans, a rare, non-atherosclerotic inflammatory vasculitis with segmental obliteration of the small and medium sized blood vessels, mostly affecting men <50 years and predominantly among smokers. However, nicotine in any form has shown an association [270]. In the sensitivity analysis, only including non-current smokers were included. Reflecting on this now, it might have been more correct to also exclude all current nicotine users.

The case series design does not allow to draw causal conclusions, still the results point towards interesting pathophysiological mechanisms It is striking that one fifth of patients with RP also had cardiovascular disease (a definition based on questionnaire, weighting together information about antithrombotic or antihypertensive drugs, and/or a CVD diagnosis). If and in what direction, there is an association (if vibration induces a systemic vasculopathy triggering a local vasospasm in the fingers, or a local injury in the endothelial cells/small nerve fibre hypoperfusion triggers a systemic vascular effect) warrants further investigation. In

a register-based study combining diagnosis with job exposure matrix, no association between exposure to hand-arm vibrations and myocardial infarction was shown [271].

Another possible confounder for in the patient study is heredity. Risk factor for RP is to have a first-degree relative who also has RP. A variant within the genetic variant of the nitric oxide gene, NOS1, has been associated with RP in the general population [123]. In the carpenter study this question was unfortunately omitted. We do however not suspect it to be more common among the carpenters than among the painters to have a heredity for primary RP and thus we do not believe that this has aggravated the ORs. In the patient study RP was based on clinical evaluation of a physician trained in occupational medicine. We cannot rule out that there could be some cases of primary RP among patients diagnosed with vibration induced RP, nor the fact that other medical conditions, primary presented as RP might debut years later.

# Conclusion

I. Carpenters had more adverse health manifestations in the hands than painters, indicating a relation to vibration exposure since carpenters are more exposed.

II. One out of ten of young carpenters showed signs of adverse health manifestations in the hands, indicating that today's work exposure is harmful.

III. More than half of the patients scored with a lower grade in the updated classification system (ICC). This calls for a change in the interpretation of severity.

IV. The neurosensory manifestations dominated the clinical picture. Cold intolerance was a very prevalent symptom among both patients and carpenters. Half of the patients and 9% of the carpenters had Raynaud´s phenomenon.

V. Patients with hand-arm vibration injury showed elevated serum levels of biomarkers indicating inflammation, endothelial injury, or dysfunction (ICAM-1, MCP-1, TM), and neuroprotection (HSP27) compared to controls.

VI. Patients with Raynaud's phenomenon showed elevated serum levels of biomarkers associated with endothelial injury or dysfunction (TM), intima hyperplasia (vWf), vaso- or neuroprotective compensatory mechanisms (CGRP) and apoptosis (caspase-3) compared to patients without RP.

VII. Serum levels of TM, vWf, HSP27 and caspase-3 were also associated with the severity of Raynaud´s phenomenon, while CGRP was associated with the severity of the neurosensory injury.

# Clinical implications

Detection of early injury is crucial to take action to prevent further progression to manifest injury. All carpenters handling vibrating tools within the construction sector should have regularly medical check-ups. The employers should provide employees with information about the risks and safety management already at the beginning of the employment. If painters handle oscillating grinding, vibration exposure need to be assessed also for painters. Both carpenters and painters should also be checked for ergonomically demanding workload, e.g., hand-intensive work tasks.

Since the symptom cold intolerance was assigned an initiating symptom among carpenters and it was frequent among both carpenters and patients, this symptom should be given more attention in the medical screening programs and should be included in the grading of severity. Clinical examination should include examination of both small and large fibre affection, e.g., impaired perception of touch and temperature might pass undetected for the exposed individual until tested clinically.

There is a need for well-defined grading system. Grading the severity of the neurosensory component of injury with the ICC would result in lower gradings than with SWS, hence the current opinion for medical advice and worker's compensation claims need modification. The SWS has been criticized for its unclarity and impreciseness, but the updated ICC might be too effective in sorting out individuals with injury. Thus, there is a need for timely and more precise diagnostic tools. Biomarkers could hopefully be used in detecting early injury before it is manifest and detectable in the clinical examination. By understanding pathophysiological mechanisms better this can enhance the development of therapies e.g., treatment with desensitisation programs for cold intolerance [119]. The results could also open for future pharmacological treatments.

# Prospect considerations

There are still many questions to be explored about the association between biomarkers and the severity of injury, e.g., do serum levels continue to rise, or is there a depletion after a while? And how does individual susceptibility affect? Associations between individual factors i.e., gender, age, and comorbidity e.g., primary RP, diabetes neuropathy, cardiovascular diseases should be addressed in future research. Many of the biomarkers analyzed can reflects inflammation, vasculo-or neuropathy in general. One fifth of patients with vibration induced RP stated such a diagnosis or had medication indicating cardiovascular disease. Do the elevated levels of biomarkers in patients with hand-arm vibration injury reflect a localized endothelial injury or a systematically distributed vasculopathy? The association between elevated biomarkers and structural changes in experimentally designed studies or studied in skin or nerve biopsies and with nailfold capillaroscopy could be explored further.

The association between cold intolerance small fiber neuropathy, CGRP and the thermoreceptors, e.g., TRPA1 and TRPV1 are highly interesting and warrants further investigation. Furthermore, the interplay between biomarkers has to be addressed further, e.g., treatment with CGRP has been shown to significantly decreased the expression of caspase-3 [272] and stimulating the TRV1 receptor via capsaicin lead to CGRP release and improvement in nerve function [273]. Today there is no special treatment for patients with diffuse neuropathy. With gained insight into the pathophysiological mechanisms behind the neurosensory and vascular components, it would be possible to develop specific therapies.

The exposure assessment is complex and there is a need to address separately, e.g., health effects from ultrafine vibrations and impact tools, and the prognosis in individuals exposed to short but extreme magnitude of vibration.

# Gender perspectives

Most workers exposed to hand-arm vibration are men. Highly exposed occupations e.g., the construction sector, demolition and concrete workers are male dominated sectors. Exposed female workers are found in higher prevalence among e.g., gardening and dentistry. In an endeavour to involve more women in male dominated occupations, companies, occupational health care services and regulatory boards must be aware of how to interpret the results from medical check-ups and risk assessments. The scientific background of the dose-response relationship relies on epidemiological studies mostly conducted on male workers. Hence female workers exposed to hand-arm vibration needs to be separately addressed in future research.

# Ethical considerations

Studies in this thesis involves human participants. The study protocol was approved by the Regional Ethics Board in Lund, Sweden (No. 2015/875 and No. 2018/15). All participants gave informed consent to participate in the study before taking part.

In the field study the participants were examined at their workplaces in their usual surrounding, with a shortened version of the routine and standardized procedures for examination hand-arm vibration injury. Little or no distress or risk was therefore expected on the examination.

Patients were examined at the clinic according to standardized procedures for examining hand-arm vibration injury. The only difference was if they agreed to participate, the collection of blood sample. The control group were examined at their workplaces with testing for perception of touch with Monofilament and blood sample collection. A skilled nurse took the blood samples with no expectations to cause any considerate harm or side effects.

Ethical considerations are that deviating results on clinical examination might initiate worrying among participants. Maybe they have been unaware or ignoring problems from their hands (and not wanting to tell their boss). Since no complete medical investigation was performed, participant with deviating results were informed to contact their general practitioner or occupational health service for follow up. There was an agreement with the heads of the companies before starting the project that follow up trough occupational health service should be available.

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