**How the study was conducted**

The present study received ethics approval from the ethics committee at the Faculty of Medicine, Philipps-University Marburg (Ref 103/02). At the end of the recruitment period it was entered in the German Clinical Trials Register (Deutsches Register für klinische Studien, www.drks.de; registration number: DRKS00010155). The study was funded by the Department of Anesthesia and Intensive Care Therapy, Philipps-University Marburg.

Patients who met all inclusion criteria and to whom none of the exclusion criteria applied received written and oral information and were then invited on the day before their operation to give their written consent to the planned study. The following inclusion criteria were defined:

- Elective surgical procedure
- Planned venipuncture before administration of anesthesia on the dorsum of the hand, examination of the vein found it in sufficiently good condition for a 17G venous cannula to be inserted
- No language barrier—able to communicate in German

Exclusion criteria:

- Raynaud’s disease
- Lidocaine intolerance or allergies
- Skin changes, such as hematomata, scars, rashes, or infections on the dorsum of the hand.

Patients were included in the study over five time periods of 3–6 months, starting in May 2005. Recruiting 450 patients took a total of 42 months. Five anesthetists in sequence undertook the venous cannulation.

On the evening before and the morning of their surgery, patients received oral premedication of dipotassium clorazepate 20 mg. Patients’ anesthesiologic preparation and continuation of patients’ own medication followed current recommendations (e1, e2). After arriving in the anesthesia preparation area, routine monitoring measures were initiated—consisting of electrocardiography, pulse oximetry, and oscillometric blood pressure monitoring. Patients were warmed up in order to prevent intraoperative hypothermia (e3).

The following items were made readily available for each patient:

- One venous cannula each of 17G (white) and 20G (pink)
- Alcohol-based disinfectant spray containing ethanol and isopropanol
- Vapocoolant spray (alkane mixture consisting of propane butane, and pentane)
- 0.5 mL of lidocaine 2% solution in a 2 mL syringe with 27G needle attached
- Alcohol-based disinfectant swabs
- I.V. dressing

According to the scheduled procedure, the side of the venipuncture was decided on and the material was prepared on that side. In order to collect data on the duration of the subsequent intervention, the time was measured after the preparations had been concluded (collating the materials and connecting the patients to the monitors). Patients were asked to turn their head to the side opposite the site of the venipuncture and to focus their gaze there, in order to prevent them from observing the procedure. A third observer monitored the patient’s direction of gaze during the subsequent procedure and also the standardized communication of the doctor performing the venipuncture with the patient. After placing the tourniquet, the anesthetist identified a vein on the dorsum of the patient’s hand. This site was disinfected by using and alcohol-impregnated swab, so as to ensure the hygienic preparation of the venipuncture site (e4). Only then, immediately before the following measures, did the anesthetist open an envelope (sealed, non-transparent), which included information on the patient’s group allocation. Local anesthetic pre-treatment of the venipuncture site (vapocoolant spray, lidocaine injection, no further measure as control group) and the size of the venous cannula were defined in accordance with the factorial study design. Randomization had been done before the actual study, by staff not involved in the venipuncture, who used random numbers (www.random.org).

The subsequent communication with the patient was bound by strict rules. After the randomization envelope had been opened, the following announcement was addressed to the patient: „You will shortly feel cold on the dorsum of your hand.“ Patients in the lidocaine group and the control group then had alcohol-based disinfectant sprayed on to the dorsum of their hand. Patients in the cryoanesthesia group, by contrast, received a puff of the freezing spray. This was administered from a distance of about 5 cm, until the venipuncture site was covered in a white mix of frozen moisture from skin and environmental air. The duration was 1–2 seconds, with a maximum of 3 seconds.

After the cold stimulant had been applied (alcohol spray or vapocoolant spray), the next standardized communication to the patient was issued: „I am now starting the venipuncture.“ In patients in the cryoanesthesia group and the control group, the venipuncture was started then. Patients in the lidocaine group, however, first received an intradermal injection of 0.1–0.3 mL of the lidocaine 2% solution by using the 27G syringe. Immediately afterwards, venipuncture was undertaken and the venous cannula inserted, but no further announcement was made.

When the first venipuncture had been completed, the time was stopped, independently of whether the procedure had been successful or not. Before an i.v. dressing was applied—that is, while remaining ignorant about the success of the procedure—patients were invited to rate their subjective discomfort throughout the entire procedure. During the initial consultation, this approach had been discussed with the patient, and the use of the 11-point numerical rating scale (NRS) had been practiced. This was intended to ensure that all problems noted during the preparations—for example, tightness of the tourniquet, impact of the cold and burning pain, but also pain at the venipuncture site—were included in the overall assessment.

The invitation to participants to rate their experience using the NRS was communicated by using a standardized phrase: „Please rate how uncomfortable the venipuncture procedure was for you just now. Pick a number from 0—no discomfort—up to 10 for intolerable discomfort.“ Only after the rating had been performed, the i.v. dressing was applied or another cannulation attempt was initiated where required.
All patients were followed up with regard to local adverse effects of the venipuncture or the preparations undertaken to this end until they were discharged from hospital.

**Study objective and clinical endpoints**

The study aimed to investigate the effects of two methods of local anesthetic pre-treatment of the venipuncture site before inserting venous cannulas of different sizes. On the one hand, the focus was on evaluating the entire proceedings of the venipuncture (including prepping the patient) and, on the other hand, on the result of the venipuncture. The two aspects cannot be separated as a venipuncture—even if not experienced as stressful by a patient—is always accompanied by additional discomfort if it has to be repeated because it failed the first time. Therefore, both aspects (subjective discomfort of the patient and the rate of failed venipuncture attempts) were equal primary endpoints of the study. The patients scored their experience in an 11-point NRS from 0–10; rates of failed venipuncture attempts were collected by means of documenting of the venipuncture result. Process times (duration from applying the tourniquet to the completion of the first venipuncture attempt) as well as possible local adverse effects of the local/cryoanesthesia were secondary endpoints.

**Sample size calculation**

Of the two primary endpoints, the rate of failed venipuncture attempts as a dichotomous endpoint necessitates a greater requirement for an adequate number of participants, and the sample size calculation was therefore based on this. Based on older publications (1, 8, 10) the researchers estimated on the basis of weighted means the increase in the rate of failed venipuncture attempts, especially after lidocaine injection, at 16 percentage points, which was confirmed by a subsequent study (16). A two-tailed chi-square test can be used to confirm such a difference (effect size $\omega = 0.30$) for an alpha error of 5% with a power of 90% and 141 patients per group, if independence of the cannula size is assumed and therefore the failed venipuncture rates for both cannula sizes are combined. Because of expected dropouts the sample size was increased to 150 patients per intervention. The large number of 450 patients made it possible with regard to the second primary endpoint to detect small scoring differences on the NRS in the range of 1.27 points. This assumes that the standard deviation of the NRS scores is twice as high as the difference between groups. The Tukey-Kramer (all pair) test achieves a power of 90% in this setting and also allows a Bonferroni correction of the alpha error to 2.5%. Consequently, the entire study was of sufficient power to verify the results of earlier studies with regard to the rate of failed venipuncture attempts and simultaneously show these as a clinically relevant improvement in patients’ scores on the NRS. Sample size calculation was performed using PASS 2002.

**Definition of clinical relevance**

In different studies, NRS differences between 0.9 and 1.3 points were defined as clinically relevant (17–19) on a visual analog scale (VAS) of 10 cm. In the named studies, this is the order of magnitude of patients’ ability to discriminate a pain sensation as slightly lower or slightly higher than in a reference stimulus by using this instrument. For the clinical interpretation of the results, the results on the VAS were applied to the NRS used in this study, to simplify the results; a difference of one NRS point was defined as clinically relevant.

**Statistical evaluation**

The plan for the statistical evaluation prospectively set out in the study protocol comprised the global analysis of the rate of failed venipuncture attempts with a chi-square test between the 3 therapeutic groups (cryoanesthesia, lidocaine injection, and controls). In case of a significant effect at the 5% level, Fisher’s exact test would then be used as a post hoc testing method to look for the source of the unequal cell frequencies. In the patients’ evaluation using the NRS, the initial step was to globally search for differences between the 3 treatment groups and the influence of the cannula size by using a three-factorial analysis of variance (ANOVA). Where the significance level adjusted to 2.5% had not been reached, the Tukey-Kramer (all pair) test was used for the specific effects of the cannula sizes on the one hand and the pre-treatment of the venipuncture site on the other hand. Secondary endpoints were analyzed descriptively. In order to take into consideration that the NRS scores are merely ranked scaled data, Figure 2 shows the box plots of the scores and the Table the corresponding arithmetic means and standard deviations. The software package JMP 9.0.1 (SAS-Institute, Cary, NC, USA 27513) was used for all statistical analyses.