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CONTINUOUS AND ACCURATE MEASUREMENT OF INTRAVESICAL VOLUME USING VIDEO-CYSTOMETRY.

Hypothesis / aims of study

Cystometry in mice is a key technique to study bladder physiology in the lab. Since Maggi et al. developed it in 1986 (1), the technique has been used in nearly every urology laboratory. However, it is difficult to master. Inter-animal and even intra-animal variability is large, variability between different researchers and different research groups is even larger and artefacts are more the rule than the exception.

An important shortcome of cystometry in mice is the lack of adequate volume measurement. Residual volume and maximum bladder capacity are two parameters we rely on the most when treating urological patients, and this is currently not reliably feasible during continuous cystometry in mice. For convenience, residual volume is often assumed to be negligible. While this might be true in physiological conditions, in pathological models things could be different.

A second drawback is the lack of information regarding the bladder outlet, since EMG recordings of the urethra in mice are difficult to obtain.

Our aim is to develop a method for non-invasive bladder volume measurement during cystometry, while providing information on the bladder outlet.

Study design, materials and methods

Female Bl6/J mice of 12 weeks old were used for the experiments. Under urethane anesthesia (1.2g/kg SC), a PE-50 catheter was implanted in the bladder dome and the animals were installed in a Bruker Skyscan 1076 micro-CT one hour after catheter implantation.

Continuous cystometry was performed, at an infusion rate of 20µl/min. Instead of normal saline, a 50% solution of iodine contrast (iomeron 250) in saline was infused intravesically. During cystometry, continuous fluoroscopy was performed, at 1.5 frames per second, 100ms exposure, 50kV and a pixel size of 35µm. First, we allowed the animal 30 minutes of habituation, then, 30 minutes of recording was performed and finally, the catheter was disconnected and all fluid draining from the catheter was captured in a microcentrifuge tube and weighed.

The resulting fluoroscopy movies were analyzed using the Fiji distribution of imageJ. In each movie, a region of interest was drawn around the bladder, and the upper thigh was used as a background region to correct for X-ray source instability. Average pixel intensity in the bladder was then plotted against time. Filling phases were analyzed for linearity, pixel intensity was converted to volume since infusion speed and intensity of the empty bladder are known. Maximum volume and residual volume was measured from the traces and voided volume calculated. Bladder volume at the end of the experiment was compared to the weighed volume in the microcentrifuge tube.

Results

Average pixel intensity in the bladder rises linearly during bladder filling (average $R^2 = 0.995$), and measurements are reproducible (average intra-sample variability ± SD = 1.6% ± 1.4%).

Since volume infusion is constant (20µl/min), and the intensity at empty bladder volume is known, intensity values can be converted to volume (fig 1).

Median difference between weighed and calculated volume at the end of the experiment was 5.2% (fig 2).

The average maximum volume was 144µl, average voided volume was 77µl, average residual volume was 67µl (fig 3).

Average intra-animal difference in residual volume is 23µl (34% difference between the lowest and the largest residual volume in one animal)

In addition to volume measurement, the X-ray images provide a detailed view on the bladder, the bladder neck and the urethra. Qualitative information is easily obtained, quantitative data has not yet been analyzed (fig 4).

Interpretation of results

We proved that volume can be measured from 2D images, using contrast intensity to obtain a 3rd dimension. To our knowledge, this is the first report to describe such a technique. Currently, no consensus about the optimal technique for residual volume measurement exists. We found that emptying the bladder by disconnecting the infusion catheter and letting it empty by gravity only, was sufficient to remove all intravesical content as could be visually confirmed under fluoroscopy. However, capturing the droplets is still prone to errors and residual volume can only be measured once during an experiment. Volume measurement using video-cystometry and weighing of the residual volume is comparable, with only 5% median difference.

Second, we showed that residual volume in mice is often higher than expected. Since urethane anesthesia was used, these findings can not be extrapolated to all mouse cystometry, but it is clear that bladder pressure traces can be undistinguishable between animals, while they have a large difference in bladder volumes. We also showed that within the same animal, residual volume can fluctuate over time. Video-cystometry can provide a complete overview of bladder volume throughout the whole experiment and will allow us to test the effect of drugs on residual volume 'on-the-fly'.

Third, the possibility to observe the motions of the bladder and the bladder neck can provide new insights into bladder function.

Concluding message

Video-cystometry can easily, reliably and non-invasively measure intravesical volume in mice at any time. The technique can easily be used to improve cystometry measurements in any laboratory where fluoroscopy- or CT-equipment is available. In addition, anatomical information can aid in the interpretation of the results.

Post-void residual volume in mice under urethane anesthesia is much higher than previously described and should not be neglected.

Figure 1:

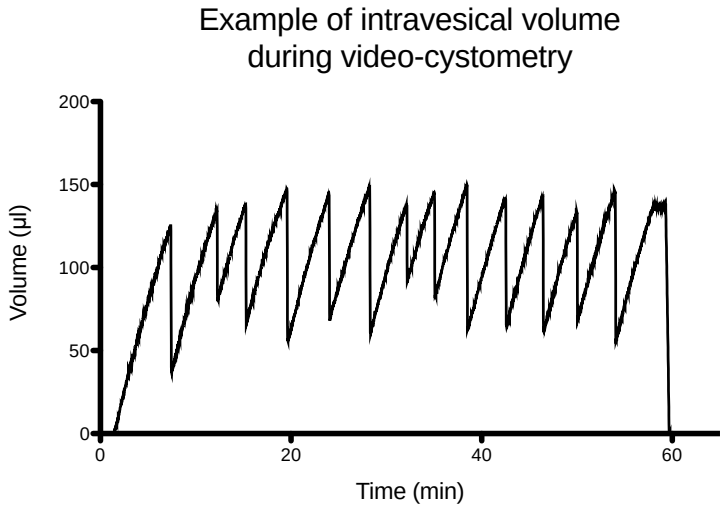


Figure 2:

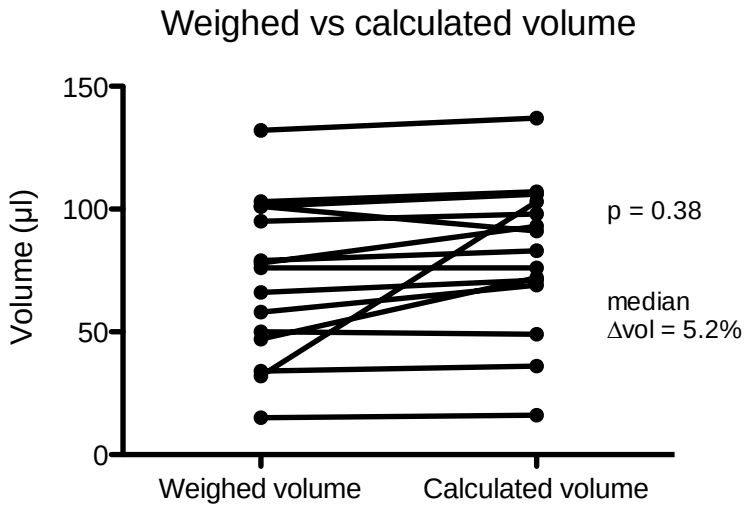


Figure 3:

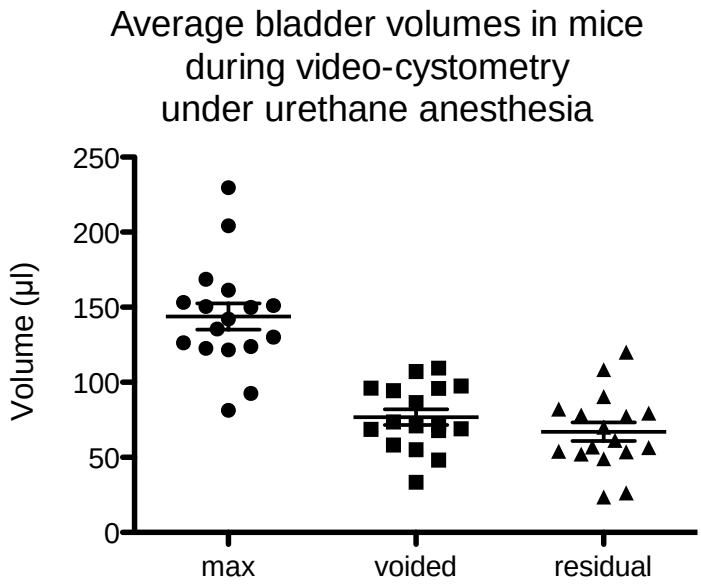
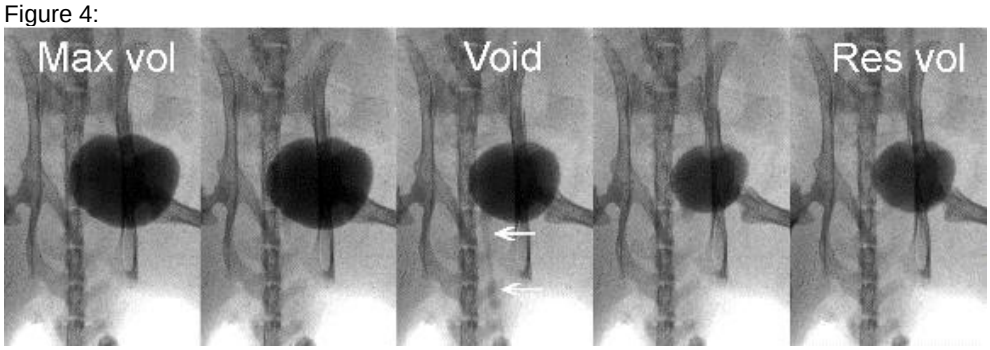


Figure 4:



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Disclosures

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