Effects of hypoglossal nerve block and electrical stimulation of the thyrohyoideus muscles on position of the larynx and hyoid apparatus in healthy horses

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Objective—To determine the effects of hypoglossal nerve block and electrical stimulation of the thyrohyoideus muscles on position of the larynx and hyoid apparatus in resting horses.

Animals—16 healthy horses that underwent hypoglossal nerve block and 5 healthy horses that underwent electrical stimulation of the thyrohyoideus muscles.

Procedures—Horses underwent bilateral hypoglossal nerve block or electrical stimulation of the thyrohyoideus muscles. Positions of the basihyoid bone, ossified part of the thyroid cartilage, and articulations of the thyrohyoid bones and thyroid cartilage were determined in radiographic images obtained before and after performance of hypoglossal nerve blocks or during thyrohyoideus muscle stimulation. Radiographic images were obtained with the heads of horses in neutral (thyrohyoideus muscle stimulation) or neutral and extended (hypoglossal nerve block) positions. Radiographic images of horses obtained after performance of hypoglossal nerve blocks were also evaluated to detect dorsal displacement of the soft palate.

Results—Hypoglossal nerve blocks did not induce significant changes in the positions of evaluated anatomic sites in radiographic images obtained in neutral or extended head positions. Hypoglossal nerve block did not induce dorsal displacement of the soft palate in horses at rest. Bilateral thyrohyoideus muscle stimulation induced significant dorsal movement (mean ± SD change in position, 18.7 ± 6.8 mm) of the ossified part of the thyroid cartilage; rostral movement of evaluated anatomic structures was small and not significant after thyrohyoideus muscle stimulation.

Conclusions and Clinical Relevance—Bilateral electrical stimulation of the thyrohyoideus muscles in horses in this study induced dorsal laryngeal movement. (*Am J Vet Res* 2013;74:784–789)

The hypoglossal nerves and the rostral hyoid muscles are important for swallowing and maintenance of nasopharyngeal stability in horses during exercise.[1–7] In mammals, laryngeal elevation is important for airway protection during the pharyngeal (second) phase of swallowing; such laryngeal elevation assists laryngeal closure via apposition of arytenoids and the base of the epiglottis and aids diversion of food into the esophagus.[8–11] This protective mechanism is mediated via activity of the hypoglossal nerves, which innervate the hyoglossus, styloglossus, genioglossus, and geniohyoideus muscles. Those muscles attach to the hyoid apparatus: hyoglossus muscles to basihyoid and thyrohyoid bones, styloglossus muscles to stylohyoid bones, genioglossus muscles to basihyoid and ceratohyoid bones, and geniohyoideus muscles to the lingual processes of the basihyoid bone. The hyoid apparatus suspends the larynx from the petrous parts of temporal bones. The hypoglossal nerves also innervate the thyrohyoideus muscles, which pull the larynx rostrally and dorsally and the base of the tongue caudally via attachments to the thyrohyoid and basihyoid bones.[12–14]

ABBREVIATION

DDSP	Dorsal displacement of the soft palate

The thyrohyoideus muscles are important for maintenance of nasopharyngeal stability in horses during exercise. Bilateral resection of thyrohyoideus muscles induces DDSP during low-speed exercise,[3] and injection of local anesthetic drugs adjacent to the distal aspects of the hypoglossal nerves induces DDSP during high-speed exercise.[2]

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The effects of stimulation of thyrohyoideus muscles and dysfunction of hypoglossal nerves on the larynx and hyoid apparatus position in horses have not been investigated, to the authors' knowledge. The purpose of the study reported here was to determine the effects of electrical stimulation of thyrohyoideus muscles and local anesthesia of hypoglossal nerves on the position of the larynx and hyoid apparatus in horses at rest and to determine whether local anesthesia of hypoglossal nerves induces DDSP in horses at rest. We evaluated the effects of bilateral thyrohyoideus muscle stimulation. We selected the thyrohyoideus muscles for stimulation because replacement of the action of that muscle with a prosthetic suture affects nasopharyngeal stability during exercise.[15,16] We hypothesized that local anesthesia of both hypoglossal nerves at the level of the ceratohyoid bones would result in caudal movement of the larynx but would not result in DDSP at rest and that electrical stimulation of thyrohyoideus muscles would result in dorsal movement of the larynx in horses at rest.

Materials and Methods

Animals—Sixteen healthy adult horses (12 Thoroughbreds, 2 Standardbreds, 1 warmblood, and 1 Thoroughbred cross; 3 sexually intact males, 5 geldings, and 8 mares; mean age, 8.7 years [range, 3 to 20 years]) with no history of upper airway disease and unremarkable resting endoscopic examination findings underwent local anesthesia of hypoglossal nerves for determination of the effects of that nerve block on laryngeal position. Five additional horses (4 Thoroughbreds and 1 Quarter horse; mean age, 7.4 years [range, 4 to 15 years]) with no history of upper airway disease and with unremarkable resting endoscopic examination findings underwent placement of electrodes for electrical stimulation of thyrohyoideus muscles to determine the effects of such stimulation on laryngeal and hyoid apparatus position. Horses had unremarkable physical examination findings and were athletically fit. Study procedures were approved by the Cornell University Institutional Animal Care and Use Committee.

Hypoglossal nerve block—Right and left hypoglossal nerve blocks were performed lateral to the ceratohyoid bones.[2] Briefly, horses were placed in stocks in a standing position and lightly sedated with xylazine (0.3 to 0.6 mg/kg, IV). Following aseptic preparation of skin, a 50-mm stimulating needle[a] was inserted in the intermandibular space perpendicular to the skin and advanced medial to the pterygoid muscle and lateral to the ceratohyoid bone approximately 1 cm caudal to the lingual process of the basihyoid bone. This needle was attached to a peripheral nerve locator device[b] (initial output settings: amperage, 2 mA; frequency, 2 Hz; pulse duration, 0.15 milliseconds). The needle was advanced until twitching retraction of the tongue was observed. Advancement of the needle toward a hypoglossal nerve was continued as the output current was reduced until the tip of the needle was < 1 mm from the nerve. A test volume (0.5 mL) of mepivacaine was injected; if there was no resistance to injection and tongue twitching stopped following injection of the test volume of mepivacaine, then an additional 2.5 mL of mepivacaine was injected to block the hypoglossal nerve.[17] The procedure was repeated for the contralateral hypoglossal nerve.

Lateral laryngeal radiographic images of horses were obtained before and 30 to 45 minutes after performance of hypoglossal nerve blocks. Dorsal displacement of the soft palate in horses was detected via observation of the soft palate dorsal to the epiglottis in radiographic images. Muzzles were placed on horses to prevent eating until tongue function returned.

Electrical stimulation of thyrohyoideus muscles—Horses were anesthetized and positioned in dorsal recumbency. For each horse, skin was aseptically prepared and a midline incision was made over the ventral surface of the larynx. The right and left thyrohyoideus muscles were identified on the ventrolateral surfaces of the larynx. A needle[c] with an inner insulated guide wire was placed in the middle aspect of each thyrohyoideus muscle, and the tip of the needle was used to electrically stimulate the muscles. After appropriate contraction of thyrohyoideus muscles was observed during electrical stimulation, a custom-made 5F bipolar electrode[d] with a built-in electrical ground was then placed in each muscle and secured with 2-0 nylon suture. The outer contacts of the electrode were wired together to form a common ground; the central contact of the electrode was the active contact. To prevent dislodgement, electrode leads were secured to the lateral aspects of the thyroid cartilage with 2-0 nylon suture. Electrode leads were tunneled subcutaneously and attached to a custom connector[e] placed subcutaneously; the connector was used to electrically stimulate thyrohyoideus muscles with an external programmable stimulator.[f] Horses received trimethoprim-sulfadiazine (30 mg/kg, PO, twice daily) and phenylbutazone (1 mg/kg, PO, twice daily) for 5 to 7 days. Horses were examined daily to detect signs of pain, soft tissue swelling, or dysphagia.

Electrical stimulation testing of thyrohyoideus muscles was performed 2 to 6 weeks after electrode placement. Electrical stimulator settings that induced maximal laryngeal movement (biphasic square-wave pulse waveform; frequency, 50 Hz; pulse duration, 2 milliseconds; amplitude, 4 to 10 V; microphase gap, 5 microseconds) were used; these settings had been determined during preliminary experiments.

Determination of laryngeal position—The effects of electrical stimulation of thyrohyoideus muscles and local anesthesia of hypoglossal nerves on laryngeal position in horses were determined with a previously validated radiographic reference system.[2,15,18] The positions of the following 3 laryngeal sites of interest were determined in lateral radiographic images obtained before (control position) and during electrical stimulation of thyrohyoideus muscles or local anesthesia of hypoglossal nerves in horses: the caudal aspect of the basihyoid bone, the ossified part of the body of the thyroid cartilage, and the articulations of the thyrohyoid bones and the thyroid cartilage. All radiographic measurements were corrected for magnification by use of reference pins of known length (50 mm). These pins were placed on the lateral aspects of the left and right masseter muscles of horses before radiographic images were obtained. Magnifications of the pins on the skin of left and right masseter muscles were determined, and the magnification of midline structures was determined via calculation of the mean value of the pin magnification values.[19] The positions of the 3 laryngeal sites of interest (basihyoid bone, ossified part of the thyroid cartilage, and articulation of the thyrohyoid bones and the thyroid cartilage) were determined in a rostrocaudal plane parallel to the ventral aspect of the horizontal rami of the left and right mandibles and in a dorsoventral plane parallel to the caudal aspect of the vertical rami of the left and right mandibles **(Figure 1)**. For the rostrocaudal plane, positive and negative values indicated movement of structures in rostral and caudal directions, respectively. For the dorsoventral plane, positive and negative values indicated movement of structures in dorsal and ventral directions, respectively. This method allowed determination of repeatable measurements with minimal confounding attributable to obliquity of radiographic images.[15]

Horses were lightly sedated with xylazine (0.3 to 0.6 mg/kg, IV), and radiographic images were obtained with their heads in a neutral position. Additional radiographic images were obtained for 8 of the horses with their heads in an extended position (angle between the intermandibular space and the ventral aspect of the neck, 110°) following local anesthesia of hypoglossal nerves to determine the effect of head extension on laryngeal position.[20] Radiographic images of horses were not obtained with their heads in a flexed position because changes in laryngeal position cannot be detected in radiographic images obtained with heads of horses in that position.[20] Radiographic images were evaluated, and measurements were obtained by use of a digital radiography system.[g]

Statistical analysis—A Wilcoxon signed rank test was used to determine the effect of hypoglossal nerve block on the position of each laryngeal anatomic site of interest (basihyoid bone, ossified part of the thyroid cartilage, and articulations of the thyrohyoid bones and thyroid cartilage). The effects of simultaneous electrical stimulation of right and left thyrohyoideus muscles on positions of laryngeal anatomic sites of interest were determined via the Dunnett method for multiple comparisons with a control group.[21] Statistical analysis was performed with statistical software.[h] Values of $P < 0.05$ were considered significant.

Results

Effect of hypoglossal nerve block on laryngeal position—Hypoglossal nerve blocks were performed successfully (as determined via observation of cessation of twitching of the tongue) for 15 of 16 horses. Data for the horse with an unsuccessful hypoglossal nerve block were excluded from analysis. After hypoglossal nerve block, tongue tone and function varied among horses. For most (13/15) horses with a successful hypoglossal nerve block, the tongue was flaccid and horses were unable to keep the tongue in the mouth. Two horses had partial motor function of the tongue and were able to keep the tongue in their mouth, but could not retract the tongue into the mouth after the tongue was manually extended. Tongue function of horses in this study was clinically normal approximately 2 to 3 hours after performance of hypoglossal nerve blocks. Because horses were not fed during that time, dysphagia of horses was not evaluated. No swelling or signs of pain were detected at the hypoglossal nerve block injection sites in horses following the procedure.

No significant changes in dorsoventral or rostrocaudal positions of the basihyoid bone, ossified part of the thyroid cartilage, or articulations of the thyrohyoid bones and thyroid cartilage were detected in

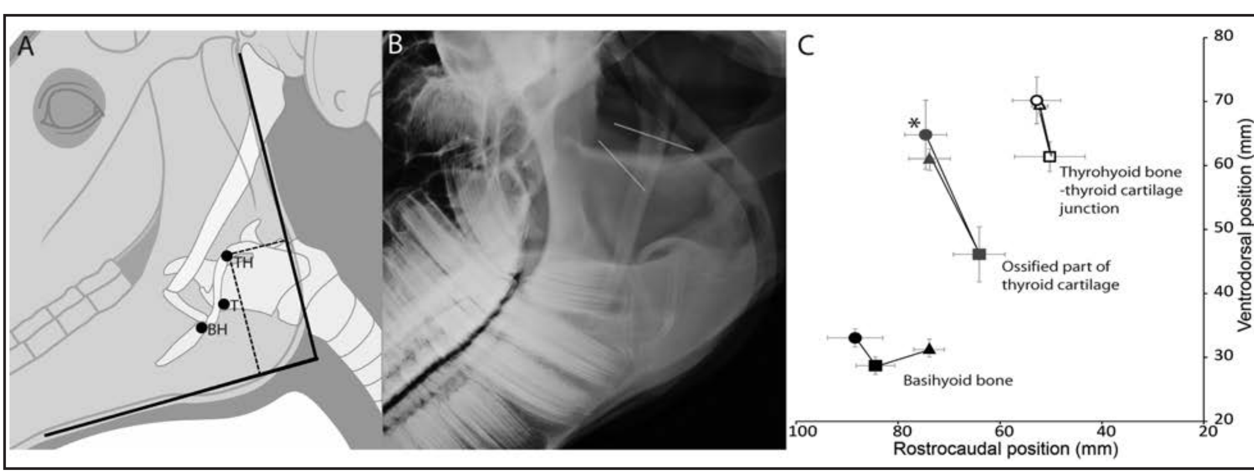

Figure 1—Illustration indicating axes used for determination of positions of the basihyoid bone (BH), ossified part of the thyroid cartilage (T), and articulation of thyrohyoid bones and the thyroid cartilage (TH) in radiographic images of horses (A); radiographic image of the larynx and hyoid apparatus of a representative horse for determination of positions of those anatomic sites (B); and figure indicating effects of electrical stimulation of thyrohyoideus muscles on positions of anatomic sites (BH, T, and TH) in 5 healthy horses of the present study and effects of placement of prosthetic sutures between the basihyoid bone and caudal aspects of laminae of the thyroid cartilage on positions of those 3 anatomic sites in 106 horses with DDSP in another study[15] (C). In panel B, notice the radiographic markers placed on the skin of right and left masseter muscle regions for determination of magnification. In panel C, mean baseline positions of laryngeal sites of horses are indicated by squares, mean positions following stimulation of thyrohyoideus muscles of horses in the present study are indicated by circles, and mean positions following placement of a prosthetic suture for horses in another study[15] are indicated by triangles. Horizontal and vertical error bars represent SE in each axis. *Data are significantly ($P < 0.05$) different from the baseline value, as determined via the Dunnett method. (Panel A adapted from Cheetham J, Pigott JH, Thorson LM, et al. Racing performance following the laryngeal tie-forward procedure: a case-controlled study. *Equine Vet J* 2008;40:501–507. Reprinted with permission.)

Table 1—Dorsoventral and rostrocaudal positions of 3 laryngeal and hyoid apparatus anatomic sites in 15 healthy horses before (baseline) and after (block) performance of bilateral hypoglossal nerve blocks.

Head position	Anatomic site	Dorsoventral position (mm)		Rostrocaudal position (mm)	
		Baseline	Block	Baseline	Block
Neutral (n = 15)	Basihyoid bone	31.0 ± 2.0	31.1 ± 2.5	99.9 ± 1.9	94.1 ± 2.4
	Ossified part of the thyroid cartilage	48.3 ± 2.1	47.1 ± 2.6	75.2 ± 2.9	69.2 ± 3.8
	Thyrohyoid bone-thyroid cartilage articulation	65.2 ± 2.6	65.8 ± 3.0	58.8 ± 2.2	54.6 ± 2.0
Extended (n = 8)	Basihyoid bone	33.0 ± 3.1	30.0 ± 3.1	94.1 ± 2.4	97.2 ± 2.8
	Ossified part of the thyroid cartilage	46.3 ± 4.2	46.3 ± 3.9	65.6 ± 4.2	68.1 ± 5.7
	Thyrohyoid bone-thyroid cartilage articulation	65.0 ± 4.4	62.5 ± 4.6	53.5 ± 2.2	57.3 ± 2.8

Data are mean ± SE.
No significant differences among data were detected.

Table 2—Changes in position of 3 laryngeal and hyoid apparatus anatomic sites in radiographic images obtained in neutral head positions for 15 healthy horses of the present study that underwent electrical stimulation of thyrohyoideus muscles and 106 horses of another study[15] that underwent placement of prosthetic sutures between the basihyoid bone and caudal aspects of laminae of the thyroid cartilage for treatment of DDSP.

Procedure	Anatomic site	Change in dorsoventral position (mm)	Change in rostrocaudal position (mm)
Thyrohyoideus muscle stimulation	Basihyoid bone	4.3 (–0.6 to 9.3)	4.0 (–9.6 to 17.6)
	Ossified part of the thyroid cartilage	18.7* (–0.2 to 37.0)	10.5 (–2.0 to 23.0)
	Thyrohyoid bone-thyroid cartilage articulation	8.8 (–4.4 to 22.0)	2.6 (–6.2 to 11.4)
Prosthetic suture placement	Basihyoid bone	3.1* (1.7 to 4.5)	–7.1* (5.3 to 8.9)
	Ossified part of the thyroid cartilage	16.2* (14.2 to 18.3)	6.0* (3.8 to 8.2)
	Thyrohyoid bone-thyroid cartilage articulation	10.3* (8.3 to 12.4)	1.5 (0.04 to 3.1)

Data are mean (95% confidence interval).
Negative values indicate ventral or caudal movement, and positive values indicate dorsal or rostral movement.
*Significant ($P < 0.05$) change in position relative to baseline position.

radiographic images of horses with neutral or extended head positions obtained after performance hypoglossal nerve blocks, compared with baseline positions of those anatomic sites (**Table 1**). Dorsal displacement of the soft palate was not detected in radiographic images obtained after performance of hypoglossal nerve blocks for any of the horses.

Electrical stimulation of thyrohyoideus muscles— Placement of electrodes in thyrohyoideus muscles was successful for all (5/5) horses. No complications or adverse effects of electrode placement (such as swelling, drainage or infection) were detected in any horse.

Data regarding the effects of electrical stimulation of thyrohyoideus muscles on laryngeal and hyoid apparatus position in 5 horses of the present study and data regarding the effects of placement of prosthetic sutures between the caudal aspects of thyroid cartilage laminae and basihyoid bones on laryngeal and hyoid apparatus position in 106 horses in another study[15] were summarized (**Table 2**; Figure 1). Electrical stimulation of thyrohyoideus muscles induced significant ($P = 0.045$) dorsal movement of the ossified part of the thyroid cartilage in horses of the present study. Minimal rostral movement of the ossified part of the thyroid cartilage was detected after electrical stimulation of the thyrohyoideus muscles; these results were not significant. Thyrohyoideus muscle stimulation did not induce a significant change in position of the basihyoid bone or the articulation of the thyrohyoideus bones and thyroid cartilage in either evaluated plane.

Discussion

Results of the present study indicated that local anesthesia of right and left hypoglossal nerves did not induce DDSP or changes in position of the larynx or hyoid bones in horses at rest. We hypothesized that local hypoglossal nerve block would induce caudal and ventral movement of the larynx at rest because ventrocaudal laryngeal movement occurs following hypoglossal nerve block in horses during exercise and is associated with DDSP.[2] Thyrohyoideus muscle contraction increases during sternohyoid and omohyoid contraction in horses during exercise and counteracts actions of those muscles[22,23]; we anticipated that decreasing the activity of rostral hyoid muscles via local anesthesia of hypoglossal nerves would allow caudal laryngeal movement and cause DDSP. This effect was not detected in horses of this study; the force produced by passive tension in those muscles may have been adequate to maintain laryngeal position in the absence of the increased contraction of sternohyoid and omohyoid muscles detected during exercise.

Results of other studies[7,12,24] indicate swallowing (during which maximal contraction of thyrohyoideus muscles is induced) is an adaptive mechanism to prevent or delay soft palate displacement in horses with naturally occurring DDSP. The etiology of DDSP in horses is not completely known, and further investigation is warranted regarding a reduction in the ability of thyrohyoideus muscles to maintain a dorsal position of the larynx during exercise.

Results of the present study indicated that electrical stimulation of thyrohyoideus muscles was safe and induced dorsal movement of the ossified part of the thyroid cartilage. Dorsal movement occurred at all 3 anatomic sites; however, other than results for the ossified part of the thyroid cartilage, the differences were not significant. These findings may have been attributable to the small sample size of horses in this study. Contraction of thyrohyoideus muscles may cause dorsal movement of the larynx via a decrease in the distance between the basihyoid bone and the larynx (depending on activities of other hyoid muscles).[25] Dorsal movement of the larynx in horses can also be induced via bilateral placement of prosthetic sutures from the basihyoid bone to the caudal aspects of the laminae of the thyroid cartilage.[15,16] The direction of change in the position of ossified parts of thyroid cartilages in horses of the present study that underwent electrical stimulation of thyrohyoideus muscles and also in horses of the other study[15] that underwent placement of prosthetic sutures was similar, although the magnitude of dorsal change in position was higher for horses of the present study. Results of the other study[15] in which the effect of a laryngeal tie forward procedure on horses with DDSP were determined also indicated that a more dorsal laryngeal position at rest during the immediate postoperative period was associated with an increased probability of racing. The amount of dorsal laryngeal movement achieved in horses of the present study during electrical stimulation of thyrohyoideus muscles was greater than that detected in horses after placement of prosthetic sutures; this finding suggested that electrical stimulation of thyrohyoideus muscles may be a treatment option for horses with DDSP. The effect of electrical stimulation of thyrohyoideus muscles in horses during exercise should be investigated.

Placement of prosthetic sutures between the caudal aspects of the laminae of the thyroid cartilage and the basihyoid bone in a horse may cause dorsal movement of the larynx by mimicking the action of contracting thyrohyoideus muscles. That surgical procedure can be used to treat DDSP in racehorses, and postoperative racing performance of horses increases with increasing dorsal position of the larynx.[15] The effect of electrical stimulation of thyrohyoideus muscles in horses with naturally occurring DDSP during exercise was not determined in the present study. However, electrical stimulation of thyrohyoideus muscles may be an alternative to placement of prosthetic sutures for the treatment of horses with DDSP because results of the present study indicated such muscle stimulation caused dorsal movement of the ossified part of the thyroid cartilage in horses. The amount of dorsal laryngeal movement required to resolve DDSP is unknown, to the authors' knowledge, and further studies may be warranted. Electrical stimulation of muscles to induce dorsal movement of larynges in horses may be beneficial because such stimulation can be selectively applied during training or racing, which might reduce complications associated with placement of prosthetic sutures (such as pulling of sutures through laminae of the thyroid cartilage).

Although we did not determine the effects of thyrohyoideus muscle stimulation on dysphagia in horses of the present study, electrical stimulation of the thyrohyoideus and geniohyoideus muscles has been used to effectively treat humans with dysphagia via elevation of the larynx.[25,26] Although uncommon, persistent DDSP can be associated with dysphagia (unpublished data); elevation of the larynx via electrical stimulation of muscles could potentially be used to treat such horses, especially those with a reduced amount of laryngeal and nasopharyngeal sensation.

The findings of this study regarding tongue function in horses after hypoglossal nerve block were consistent with findings of another study[2] in which similar volumes of local anesthetic were used to perform hypoglossal nerve blocks in horses. However, a limitation of the study reported here was the method used to determine efficacy of hypoglossal nerve blocks in horses. Although all horses had impaired tongue function and decreased tongue tone and could not retract their tongues after manual tongue extension, the amount of hypoglossal nerve function affected by local anesthesia was not determined.

Results of the present study indicated that electrical stimulation of thyrohyoideus muscles induced dorsal movement of the ossified parts of thyroid cartilages in horses. Further studies would be required to determine whether such thyroid muscle stimulation may be used to treat DDSP in horses.

a. Stimulplex Insulated Needle, B. Braun Medical Inc, Bethlehem, Pa.
b. Innervator 232, Fisher & Paykel Healthcare, Auckland, New Zealand.
c. Pajunk GmbH, Geisingen, Germany.
d. KY5, Osypka GmbH, Medizintechnik, Rheinfelden, Germany.
e. Custom Connector, Med-El Corp, Innsbruck, Austria.
f. Master-9, A.M.P.I., Jerusalem, Israel.
g. PACS, Eastman Kodak Co, Rochester, NY.
h. JMP, version 8.0.2, SAS Institute Inc, Cary, NC.

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