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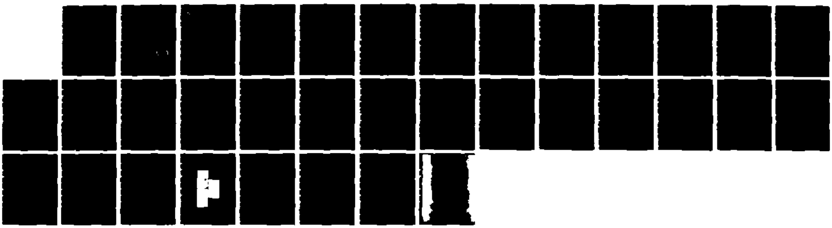

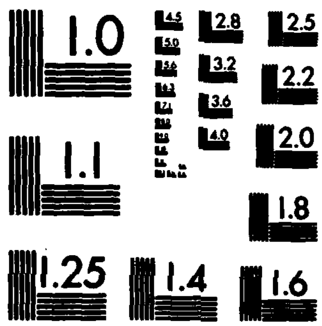

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Resolution of osseous wounds utilizing conventional radiographic techniques is dependent upon favorable angulation without superimposition of dense anatomical structures. A technique of computer subtraction utilizing sequential radiographs has been demonstrated to enhance visualization of such defects in dry skulls, but usefulness in live animals had heretofore not been demonstrated. This investigation demonstrated the usefulness of computer subtraction radiography in live animals, both error rate and diagnostic time being reduced significantly.

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SUBTRACTION RADIOGRAPHY FOR THE DIAGNOSIS OF BONE LESIONS IN DOGS*

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SUBTRACTION RADIOGRAPHY FOR THE DIAGNOSIS OF BONE LESIONS IN DOGS

ABSTRACT:

Studies with dry human mandibles have demonstrated the superiority of subtraction radiography in detecting induced lesions over conventional side-by-side comparison of two radiographs. The purpose of this study was to corroborate these findings in a live animal model. In 8 adult dogs, lesions were induced under anesthesia in mandibular alveolar bone at 14 predetermined sites. The overall probability of a lesion presence at a particular site was 1/2. Pre- and post-operative radiographs were taken with the aid of a customized occlusal template holding the film, and allowing a rigid mechanical attachment to the x-ray source. Pre- and post-operative radiographs were mounted in pairs and presented to 11 dentists for examination. A computer randomized the order of presentation and prompted the observer to examine an indicated site, soliciting a 5-level graded response, ranking from lesion definitely present to lesion definitely absent. Next, subtraction radiographs were presented on a video screen and possible lesion sites marked by circles one at a time in a random sequence. Examiner responses and decision times were recorded by computer. Diagnostic accuracy was measured by Receiver Operating Characteristic (ROC) analysis. Individual and pooled results demonstrated improved diagnostic performance for the subtraction technique ($P < .001$). Response times were also improved by the subtraction technique ($P < .0001$). Furthermore, ROC analysis showed that

the diagnostic value of radiographs can be substantially increased by digital subtraction technique resulting in an estimated 30% reduction of equivocal diagnostic decisions when subtraction images are used.

INTRODUCTION

A significant limitation in radiographic examination is the identification of subtle osseous changes indicative of pathology.[1, 2, 3] Investigations have inferred that the limitations of conventional radiography in detecting small bony lesions are largely due to the presence of structured noise.[4] Such noise consists of all anatomic features other than those of diagnostic interest. Subtraction radiography is a method by which structured noise is reduced, thereby increasing the detectability of radiographic image changes occurring over a period of time. This method has been described in detail by Grondahl, et al. and has demonstrated its potential in a study using dry human mandibles.[5] The purpose of this investigation was to employ subtraction radiography for the diagnosis of induced alveolar bone lesions in a live animal model in order to determine whether diagnostic performance could be improved over that obtained by a conventional technique.

MATERIALS AND METHODS

Eight adult mixed breed dogs* were premedicated with atropine and anesthetized (sodium pentothal induction, halothane and nitrous oxide maintenance). Several spherical radiopaque markers were cemented in small cavities prepared in the third and fourth mandibular premolars to provide fixed radiographic reference points located within the mandible. Custom cold-cure acrylic** occlusal registration/film-holder devices were fabricated so that size 2 Kodak ultraspeed dental film*** could be related to each dog's anatomy in a reproducible manner. The film holder was rigidly attached to the x-ray source and the film was exposed for 0.4 seconds at 90 Kvp and 15 milliamps.

Each film packet contained two films; one was developed immediately for on-site evaluation of geometric reproduction, the second film in each packet was stored in a light-proof container at 4° C until the end of the *in vivo* portion of this investigation. At that time all stored films were processed simultaneously to limit densitometric variation.

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* "In conducting the research described in this report the investigators adhered to the "Guide for Animal Facilities and Care" as promulgated by the Committee of the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences, National Research Council."

** Ortho Plastic; L.D. Caulk Co., Medford, DE 19963

*** Eastman Kodak Co., Rochester, NY 14650

In the mandibular premolar areas interproximal and buccal lesions were induced with a slow speed #6 round bur cooled with saline. The lesions were distributed bilaterally among the 14 predetermined possible sites (Figure 1) in a balanced, randomized design. This resulted in 52 lesions (Table 1) and 52 matched control sites distributed such that the prior probability of a lesion being present at a particular site was 1/2. It was estimated that from 12 to 40 cubic millimeters of bone were removed from each lesion. On the buccal lesions, the cortical plate was always penetrated. The dogs were allowed to regain consciousness and were returned to the animal care facility for post-treatment care. Analgesics were provided for up to two days and a soft diet was provided for seven days. All dogs were fully recovered by the end of the investigation.

The subtraction images were obtained from the preoperative radiographs by digital subtraction as described by Grondahl, *et al.*[6] Briefly, the radiographs were converted to 512 x 512 x 8 bit digital images by a TV camera interfaced with a computer controlled analog-to-digital converter, and subsequently stored in magnetic disk files. The precision of spatial registration of corresponding pre- and postoperative radiographs was monitored by analog signal subtraction via mixing of the appropriate electronic signals. To that end, the previously digitized preoperative radiograph was displayed as a positive on a video monitor, on which was superimposed the real-time negative image produced from a video camera focused on the postoperative radiograph. This resulted in real-time subtraction of the two images on the screen. By adjusting the position of the post-operative radiograph under the TV camera with the aid of a micromanipulator, the subtraction image on the monitor was brought as close as possible to null. Maintained in this position, the postoperative radiograph was then digitized and stored in another file. The result of the digital subtraction of the two images was stored in a third file. By adding a constant gray-level value of 128 to the subtraction image, relative bone loss and gain with respect to the preoperative radiograph appeared as darker or brighter areas, respectively, against the background.

Eleven practicing dentists participated in the evaluation test. They were informed about the purpose of the investigation, and that lesions were present in about half of the possible sites. The pre- and postoperatively obtained radiographs were marked accordingly and mounted in pairs on cardboard frames. The frames were given an identification number for later retrieval during the reading sessions. A light box was shielded to accept the frames and placed on a table, which also provided space for the keyboard of the computer terminal and four black and white video monitors. A computer program randomized separately for each reader and each modality the order of presentation of the radiographs or subtraction images. The room lights were dimmed and a magnifying glass was provided. Each participant read both modalities in two different sessions, which were separated by an intermission of 20 minutes. About half of the readers interpreted the conventional radiographs first, the other half started with the subtraction images. Each reader was permitted to use unlimited time to make each diagnostic decision, but the time actually taken was measured by the computer. This resulted in nominal reading sessions including training time lasting 30 to 45 minutes for the conventional radiographs, and 15 to 20 minutes for the subtraction images. At the beginning of each session written and oral instructions were given, explaining the logistics of the test procedure and the manipulations required from the readers. A test administrator initiated a trial run and let the readers practice the required manipulations. After sufficient familiarization, the actual reading session was started, and the test administrator remained present during the reading of the first few cases.

The reading session with the conventional radiographs proceeded as follows. The computer program displayed the identification number of the film-pair to be mounted on the light box. When ready, the reader pressed a key, initiating the display of the preoperative radiograph on the video monitor. The program then superimposed in an independent random sequence a circle over each possible lesion site, prompting the reader to examine the indicated site on the mounted film-pair. The observer responded by typing a number from 1 to 5 on the keyboard, rating his confidence that at the indicated site a lesion was:

(1) definitely, or almost definitely present
(2) probably present
(3) possibly present
(4) probably absent
(5) definitely, or almost definitely absent.

The response initiated the program to superimpose either another circle, or, after interrogation of all possible sites, to request another film pair. The computer recorded the choices and the time intervals required to reach the diagnostic decisions. The latter were measured from the time a circle appeared on the monitor to the time when the response was entered on the keyboard.

The reading session with the subtraction radiographs was structured similarly. However, instead of an identification number, the subtraction image to be interpreted was displayed on three different video monitors simultaneously. One monitor was set for high contrast display, another was adjusted at low contrast, and the third was left for the reader to manipulate brightness and contrast to his own preference. At the key-stroke indicating readiness of the reader, the computer program superimposed one circle at a time in a random sequence to interrogate the possible lesion sites. The rated responses and the corresponding times required in making the decisions were recorded automatically.

Immediate feedback on each decision was provided in order to sustain the readers' interest and to help them achieve and maintain stable performance. The computer program responded to each entry as follows:

decision rating	response	
	lesion present	lesion absent
1	"you are correct"	"there is no lesion"
2	"you are correct"	"there is no lesion"
3	"there is a lesion"	"there is no lesion"
4	"there is a lesion"	"you are correct"
5	"there is a lesion"	"you are correct"

DATA ANALYSIS

The diagnostic performance of the readers using both of the two radiographic modalities was evaluated by ROC (receiver operating characteristic) analysis. ROC analysis provides an index of diagnostic accuracy that is independent of extra-image decision factors and prior probability of lesion occurrence.[7] Specifically, for a diagnostic system with given discriminatory capacity, the ROC curve shows the trading relationship between the proportions of true-positive (TP) and false-positive (FP) responses, as the decision criteria to call the findings positive or negative is varied systematically. In our particular application, this graph can be assessed from the loci or points describing the relative TP and FP decisions that would be made by considering each boundary between the five examiner choices a different decision criterion. The above procedure provided four possible points that are located on a conceptually smooth curve characterizing the discrimination capacity of a particular modality. A commercially available computer program (RSCORE)[8] was used to fit an ROC curve through the four empirically obtained data points. The theoretical curve is based on the assumption that the distributions of the psychologically perceived signal strengths in the presence or absence of a lesion are normal.[7, 8] Consistent with this assumption, the data points can be plotted on double probability (binormal) coordinates**** and fitted by a straight line. The computer program provided measures of goodness-of-fit of that line and a maximum-likelihood estimate of an ROC index of diagnostic accuracy, A_z, as well as its corresponding sampling variance. The index A_z reflects the location of the entire ROC curve rather than any particular operating point thereon. A_z is defined by the area beneath the fitted ROC curve, and ranges from a minimum of 0.5 for chance performance to a maximum of 1.0 for perfect discrimination capability.

In order to summarize the performances achieved with each modality, the accuracy indices, A_z, estimated from each of the examiners' responses were either pooled or averaged. The associated standard errors were obtained from the sampling variances of the maximum-likelihood estimates given by the computer program. The statistical significance of the observed

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**** Chart Y4231, Codex Book Company, Norwood, MA 02062 difference in A_z between the two modalities, and between groups of lesion sites with comparable anatomic obscuration was tested by a paired comparison. This was possible because each reader participated in the evaluation of both modalities. A non-parametric test (sign test) was preferred in view of the limited range of A_z and the small number of readers which renders the normality assumption questionable.

The time intervals required in making decisions were averaged over all readers and all lesion sites, or groups of lesion sites with presumed similar detection difficulty. The observed averages were compared by the t-test for statistically significant differences.

RESULTS

Figure 2 shows a representative example of corresponding pre- and postoperative radiographs, and the ensuing subtraction image. The superimposed circles appeared one at a time in a random sequence over each potential lesion site. While it is nearly impossible to detect all lesions by comparing the postoperative (upper right) versus the preoperative (upper left) radiograph, the lesions are easily detected in the subtraction image (below) as dark blotchy areas. In this particular example from a right mandible, lesions were induced at sites 1, 3, 4, and 5. The bright disk-shaped artifacts in the radiographs are projections of spherical radiopaque markers serving as reference points to monitor the reproducibility of the radiographic projection geometry.

The diagnostic performance attained with each modality is shown in Table 2. A clear superiority of the subtraction technique over the conventional method of comparing radiographs is evident. At the outset of the investigation, lesion sites were grouped as shown in Table 1 based upon the presumption that members of each group would be subject to comparable obscuration due to anatomic overlay. For the conventional technique, the results indicate a definite decrease of A_z for the detection of interproximal lesions as compared to the interradicular and radicular groups ($P < .01$). No significant differences existed between groups for the subtraction technique, consistent with the premise that the source of anatomical obscuration is cancelled by subtraction.

The data also show that pooling the 11 readers' raw data, i.e., treating them as one reader by merging their rating responses, leads to a small depression of the accuracy index as compared to the average taken over the individual's indices. This is to be expected theoretically,[8] however, the small difference observed between the two summary measures attests to the relative uniformity of the decision criteria used among the different readers.

Figure 3 shows the detection performance evaluated for the total set of lesion sites. Every reader achieved a higher accuracy using subtraction images ($P < .001$). Also evident is the more uniform performance among the readers for the subtraction as compared to the conventional technique.

Figure 4 displays the ROC data points obtained by pooling the responses of each reader. Also shown are the best-fitted lines for each modality plotted on double probability coordinates. The ROC for the subtraction technique is seen to be consistently above that for the conventional technique, with respective values of A_z of 0.98 and 0.83.

A comparison of the time intervals required to decide whether at an indicated site a lesion was present or absent is shown in Table 3. In general, for each of the groupings, as well as the total pool of lesion sites, the time differences between the two modalities were highly significant ($P < .001$). The average response times observed for the conventional technique were almost four times longer and displayed approximately twice the standard errors as compared to the subtraction technique. Furthermore, the relative difficulty of detecting lesions at different sites was somewhat reflected in the times recorded for the conventional technique. The average response time for lesion sites 3 and 6, where presumably the least amount of obscuration existed, was the shortest, and was statistically different ($P < .02$) from that obtained for sites 1 and 2. In contrast, the times required in making the decisions using the subtraction technique were homogeneous among the lesion sites (analysis of variance, $P > .75$).

DISCUSSION

Previous studies with skull phantoms have indicated that subtraction radiography can improve diagnostic accuracy when compared with the conventional radiographic technique.[5] This investigation has confirmed these results in a live animal model. The clear superiority of the subtraction technique, as demonstrated in this and other studies, is critically dependent on the ability to limit geometric and densitometric variation between radiographs to be compared. However, despite the authors' best efforts these variations were, at times, quite evident and dictated the two-film packet technique. The two-film packet technique allowed the authors to continue making radiographs until an empirical on-site visual confirmation of geometric standardization could be made. Two radiographs per site at each observation interval was usually sufficient. The amount of empirically observed geometric variation over the eight week period during which radiographs were gathered appeared constant. Even a rigid registration method may, over times longer than those used in this investigation, present geometric variation problems due to normal minute changes in tooth position which may occur over time in some animals. Other researchers have used a non-rigid occlusal registration with some success,[9] although in any subject under general anesthesia, as well as any animal, the use of a non-rigid occlusal registration would likely add additional undesirable geometric variation. The method utilized in this investigation to limit densitometric variation fell short of the authors' goal of virtual elimination. Indeed, frequently the films processed in a hand developer immediately following exposure showed less densitometric variation than did the duplicate films stored and processed under more carefully controlled conditions. Fortunately the program used for subtraction radiography can compensate for densitometric variation.[10] The choice of 90 Kvp exposures was made in order to parallel clinical practice in our area. 60-70 Kvp would have produced more contrast in the radiographic films used for the conventional technique, but likely would have had little effect on the results produced after subtraction because the contrast under the latter conditions can be manipulated electronically.

The third and fourth mandibular premolar area was selected for this investigation because, in the dog, this region has sufficient lingual vestibule depth for parallel film placement and there is no interproximal contact or overlap of the third premolar with the adjacent teeth. Potential lesion sites were chosen to reflect incipient interproximal periodontal lesions without cortical plate penetration (Figure 1, Lesions 1 and 2), as well as a variety of overlaying anatomical structures for those lesions designed to penetrate the cortical plate (Figure 1, Lesions 3, 4, 5, 6, and 7). The results shown in Table 2 indicate that the diagnostic accuracy in detecting interproximal lesions (sites 1 and 2) by the conventional technique was substantially reduced as compared to the other lesion sites (A_z = .77 versus A_z = .87). This finding is in agreement with other research suggesting that lesions not involving the cortical plate are more difficult to detect (in conventional radiographs) than those lesions with cortical plate involvement.[3] Contrasting with this, the corresponding data from subtraction radiography do not show a specific association of accuracy with lesion type. Such a result of constant detection performance irrespective of anatomical context should be expected from a technique that is effective in suppressing structured noise.

In a clinial situation, a diagnostician frequently must make a decision utilizing less than conclusive evidence. In these situations a clinician is likely to skew his decision towards a diagnosis, which once made, imposes the least harm to the patient if the diagnosis is later determined to be incorrect. In an investigation as this, there was no danger to a patient in the case of an incorrect diagnosis, and the decisions were presumably based solely on the knowledge of the prior probability of lesion occurrence and the information derived from the images. The diagnostician was not restricted by clinical pressures and thus was free to express his confidence in each diagnosis by the rating scale provided. Hence, this technique permitted estimating selective points on the ROC curve from the proportion of TP anf FP decisions that would be made by choosing, in turn, each of the possible rating levels as decsision thresholds between accepting or rejecting the presence of a lesion.

Under clinical conditions, the decision thresholds or operating points on the ROC curve chosen by a diagnostician are usually not known because they vary with the particular diagnostic task, the value judgments made about morbidity and financial impact, and estimates of disease prevalence for a particular patient. However, the estimated graphs shown in Figure 4 still apply in the clinical content because they describe the possible trade-offs that can be made between correct and incorrect decisions. Expressed differently, every operating point that may be adopted by a diagnostician _must_ lie on the appropriate ROC curve for a given radiographic technique. For example, if a false-positive (FP) rate of .10 is clinically acceptable in a particular situation, subtraction radiography would attain a true-positive (TP) proportion of 0.95, as compared to the conventional technique with a TP proportion of 0.60. Or, if for some reason the false-negative (FN) decision rate must be kept small, say below 0.01, it can be seen from the scales to the left and at the top of the diagram that subtraction radiography could provide a true-negative (TN) decision rate of about 0.60, compared to a corresponding rate of 0.06 attainable with the conventional technique.

In practical setting, diagnosticians may simply withhold a definite response in equivocal situation and request further diagnostic evidence. Usually it is desirable to maintain both the probabilities of FP and FN responses below a certain acceptable level. The proportion of equivocal decisions (EV) resulting under these constraints can be estimated from the appropriate ROC curves. This follows from the fact that the probabilities of the possible responses that may be given to sites with a lesion must add up to 1.0; i.e., P(FN) + P(EV) + P(TP) = 1.0. Conversely, it also follows that the probabilities of the possible responses that may be given to sites without a lesion must add up to 1.0 as well; i.e., P(FP) + P(EV) + P(TN) = 1.0. With the aid of Figure 5 it can be seen from Figure 4 that the subtraction technique could maintain both P(FP) and P(FN) less than or equal to 0.06, while definitely sorting all lesion sites into positive or negative. Whereas to maintain P(FP) = P(FN) $\leq$ 0.10, the conventional technique would produce P(TP) = 0.60 and P (TN) = 0.45, and fail to diagnose 30% of the sites having lesions and 45% of the sites without lesions. Hence, for equal probabilities of lesion presence or absence, the conventional technique would remain equivocal on about 1/3 of the lesion sites presented. This analysis makes it clear that the observed difference of 0.15 in A_z between the two radiographic modalities is a substantial practical difference, which appears large enough to outweigh any value judgments that may be assigned to correct and incorrect diagnostic decisions.

The time intervals required in making the diagnostic decisions provided another independent assessment of the relative diagnostic utility of the two techniques. For all lesion types, these intervals were significantly shorter and more uniform using the subtraction images as compared to the conventional method. While the practical impact of this result may not be as important, it indicates that a loss of diagnostic performance due to observer fatigue is less likely to occur with the interpretation of subtraction images than with conventional radiographs.

When the ability of examiners to identify interproximal lesions using conventional radiographs was compared to subtraction images, the advantage of this technique became even more apparent. (Table 2) Such an observation supports the large body of dental literature and adds weight to Pritchard's observation that such lesions may be difficult or impossible to detect, dependent upon local anatomical factors.[12] The subtraction image is not affected by such constant, unchanging anatomical factors.

CONCLUSION

Computer subtraction images were shown to be far superior to conventional radiographic images for lesion detection in a live animal model. Diagnostic accuracy was significantly improved with subtraction radiography ($P < .001$), and the time required for diagnosis was significantly reduced as well ($P < .001$). Furthermore, ROC analysis showed that the diagnostic value of radiographs can be substantially increased by digital subtraction resulting in an estimated 30% reduction of equivocal diagnostic decisions when subtraction images are used. This technique holds great promise as a non-invasive means for accurate detection and documentation of osseous change occurring in the periodontium.

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Table 1: Distribution of Lesions

Site (from Figure 1)	Type	n
1, 2	interproximal	15
3, 6	interradicular	15
4, 5, 7	radicular	22
	total	52

Table 2. Attained Measures of Performance A_z

Site		Conventional	Subtraction
1, 2	pooled	.76	.98
	averaged	.77* (.13)**	.98 (.03)
3, 6	pooled	.88	.98
	averaged	.88 (.10)	.99 (.02)
4, 5, 7	pooled	.86	.98
	averaged	.86 (.07)	.98 (.02)
all	pooled	.83	.98
	averaged	.84 (.05)	.98 (.02)

* Significantly different ($P < .01$) from either site groups (3, 6) or (4, 5, 7).

** Numbers in parentheses represent standard error of the mean.

Table 3: Time Required to Perform Lesion Detection Task (sec.)

Site	Conventional	Subtraction
1, 2	10.19 (.40)**	2.78 (.17)
3, 6	8.69* (.37)	2.41 (.14)
4, 5, 7	9.91 (.46)	2.80 (.19)
all	9.21 (.46)	2.66 (.20)

* Significantly different ($P < .02$) from sites 1 and 2.

** Numbers in parentheses represent standard error of the mean.

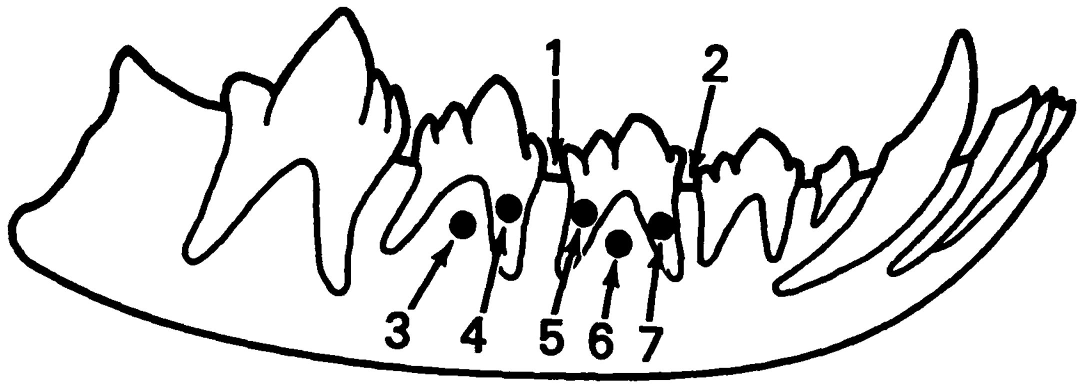

Figure 1: Buccal view of potential lesion sites in the dog mandible.

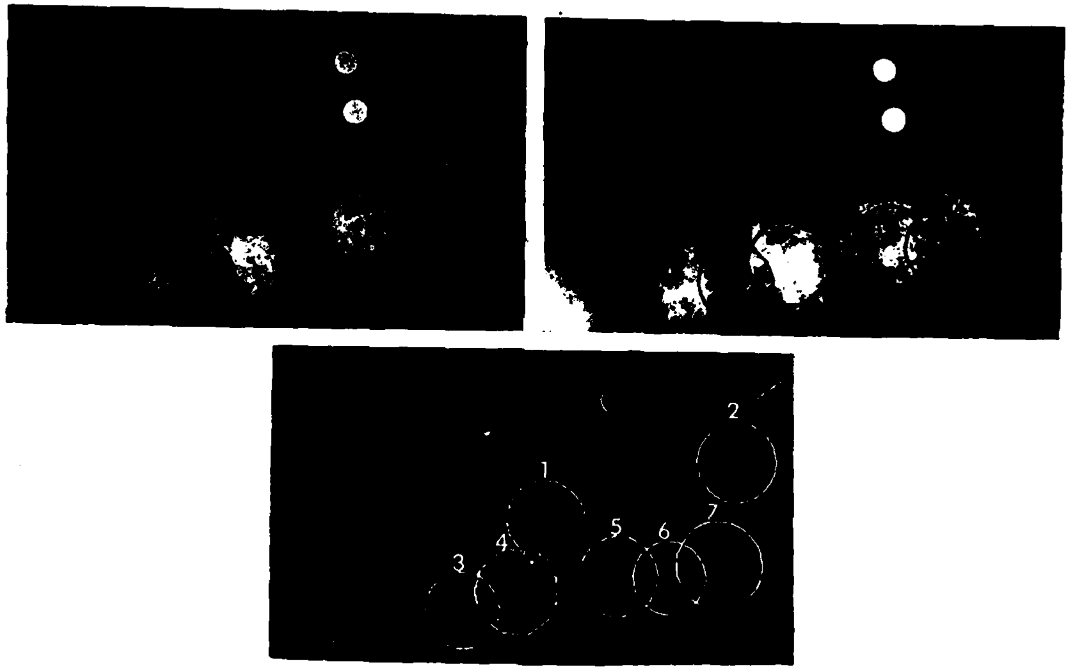

Figure 2: Representative example of corresponding pre- and post-operative radiographs and the ensuing subtraction image. *Upper left:* Pre-operative radiograph. *Upper right:* Post-operative radiograph. *Below:* The subtraction image.

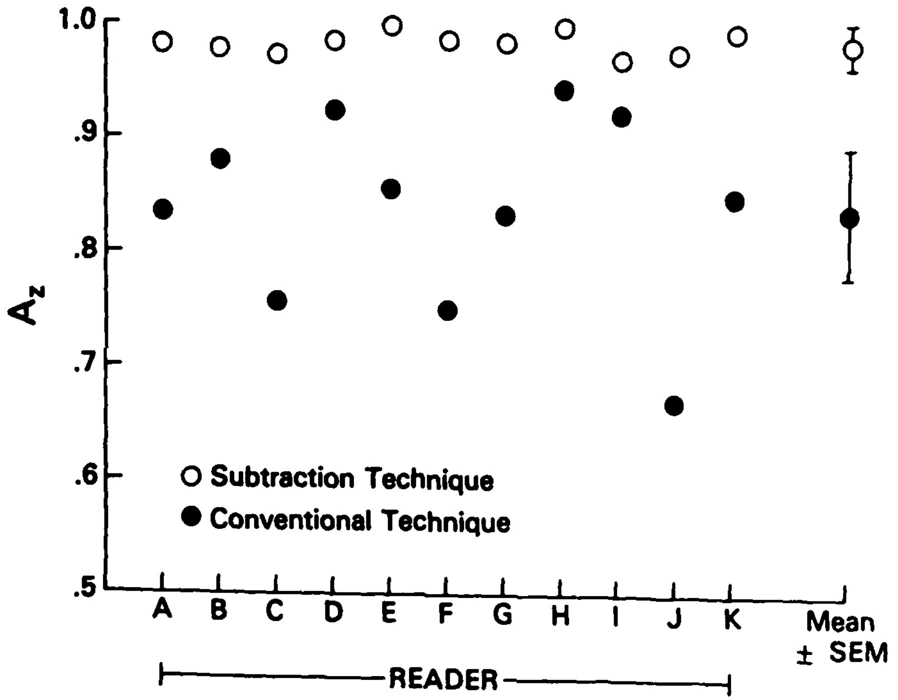

Figure 3: Reader performances, mean scores, and standard error of the mean measurements.

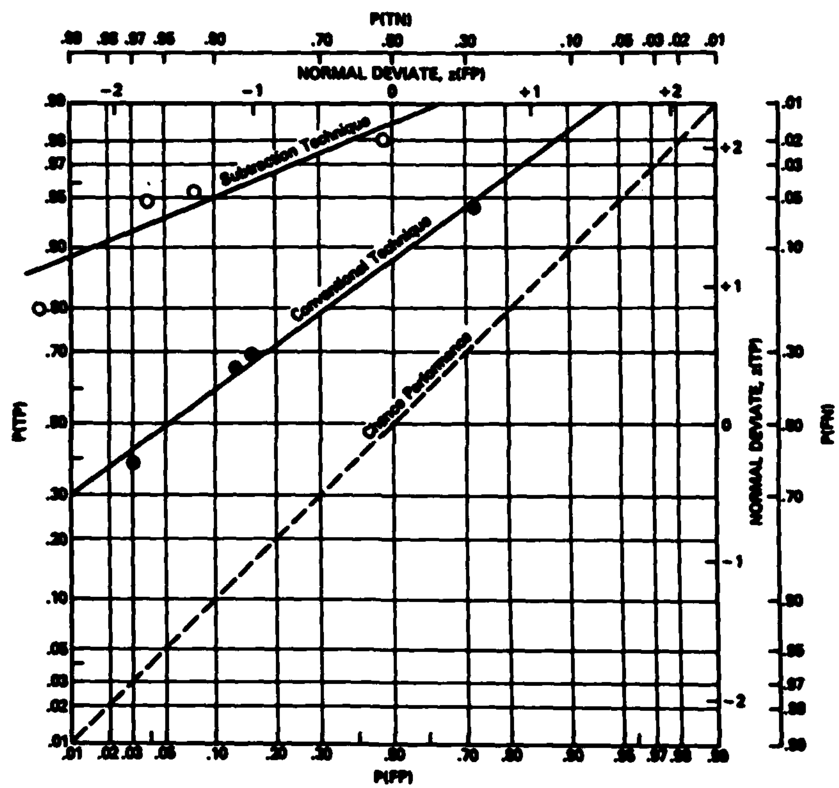

Figure 4: ROC data points and best-fitted lines for both radiographic techniques. The greater the area below a particular best-fitted line the better the performance.

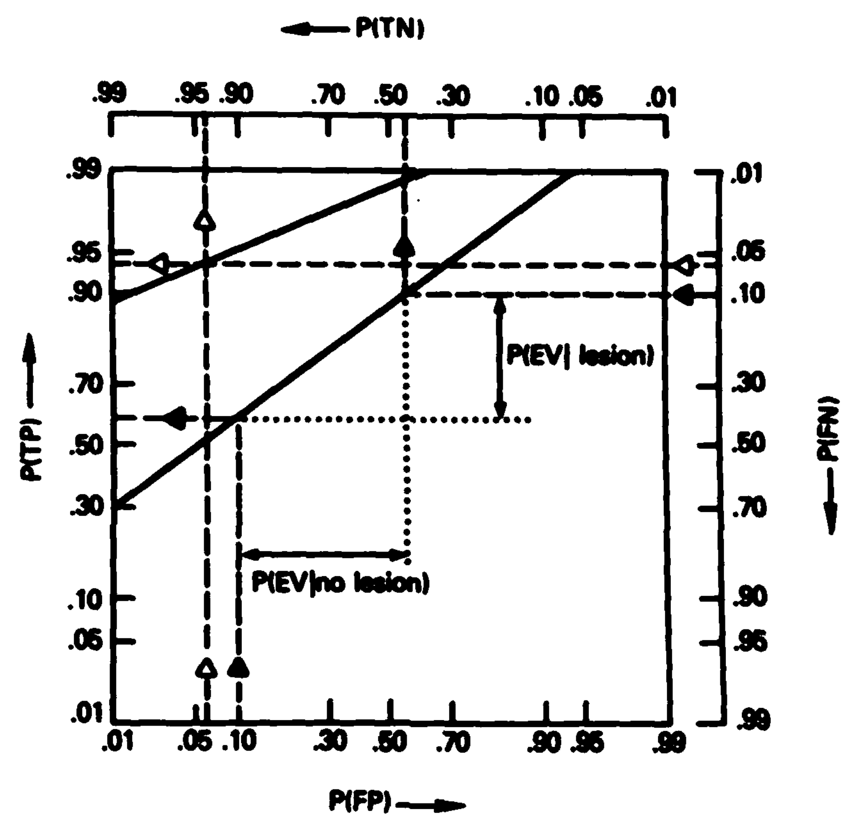

Figure 5: ROC analyses for predictions of equiovocal responses for each radiographic technique. In the first example, dashed-lines marked with undarkened arrows demonstrate the desirable lack of equivocal responses using the subtraction technique if false-negative (FN) and false positive (FP) error proportions of 6% are acceptable to the diagnostician. In the second example, the dashed-lines marked with darkened arrows define the expected proportions of undesirable equivocal responses made by diagnosticians utilizing conventional radiographic technique if acceptable FN and FP error proportions are allowed to rise to a less stringent 10%.

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