NATIONAL ANALGESIC NEPHROPATHY STUDY

PROTOCOL – PHASE I

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1. Introduction

In October 1999, the contract for the National Analgesic Nephropathy Study (NANS) was awarded by NIDDK to the Medical College of Ohio. This document is the protocol for Phase I, during which the study methods and procedures will be developed, tested, and finalized. The proposed methods and their rationale are described in detail in the original Technical Proposal and further elaborated on in our response to questions of January 11, 1999. There have been relatively few changes since the study began, and much of what appears here will therefore be a recapitulation and expansion of the proposal.

2. Background and Objectives of NANS

Based mainly on clinical evidence, supported by experimental studies and correlational data, heavy analgesic use has been implicated in the etiology of a unique nephropathy characterized by renal papillary necrosis and interstitial nephritis (analgesic nephropathy, or AN). Excessive use of analgesics is also a suspected cause of other forms of chronic renal disease. There have been a number of formal epidemiologic studies of these topics, but they have been unable to delineate the precise relationship of analgesic intake to chronic renal insufficiency because of flaws in their design. The etiology of chronic renal disease is still poorly understood, largely because its onset is insidious, with early distinctive pathological changes often obscured as the disease progresses.

A new study is both necessary and timely because (1) the number and type of analgesics in the U.S., either as single ingredient products or as combination products, is large and increasing; (2) the incidence and prevalence of analgesic-related renal disease remains unknown in the U.S.; (3) the types of analgesics linked to the development of renal disease and the

requisite amount of consumption are still unclear; (4) recent evidence has suggested the possibility that the abdominal CT scan may be a useful diagnostic tool for the identification of AN; and (5) much of analgesic-associated renal disease may be preventable.

The specific objectives of the study are as follows:

1. To determine whether non-contrasted CT among patients with end stage renal disease (ESRD) can be used with adequate precision to identify cases of AN as a distinct entity.
2. If AN can be identified, to determine which analgesics, including combination analgesics, are associated with its development, and to determine the effects of duration of exposure and dosage.
3. If AN can be identified, to determine its incidence and the proportion of the total burden of ESRD in the U.S that it represents.
4. Whether or not it is possible to identify AN as a distinct entity by CT scan, to determine the relation of analgesic use (the effects of specific drugs, duration of exposure, and dosage) to the *de novo* onset of any chronic renal insufficiency leading to ESRD.

The study will be conducted in two phases, with a possible third phase depending on the outcome of the first two. The remainder of this protocol refers to Phase I, which will be a 14 month period devoted to methodological development, including the questionnaire that will be used in Phases II and III, the CT scan parameters to be used as criteria for AN, and other aspects of the methods.

3. Study Organization

The project is being conducted under the overall direction of William L. Henrich, M.D., Professor and Chairman of the Department of Medicine at the University of Maryland School of

Medicine, who is the Coordinating Investigator, and Joseph I. Shapiro, M.D., Professor of Medicine and Pharmacology and Chairman of the Department of Medicine at the Medical College of Ohio, who is the Principal Investigator. The Slone Epidemiology Unit (SEU) of Boston University School of Medicine is the Coordinating Center; David W. Kaufman, Sc.D., Associate Director, and Samuel Shapiro, M.B.,F.R.C.P.(E), Director Emeritus, are Coinvestigators.

The study will be conducted under the direction of a nephrologist Collaborating Investigator in each of three centers: Vardaman M. Buckalew, Jr., M.D., Professor of Medicine at the Wake Forest University Baptist Medical Center in Winston-Salem, NC (WFUSM); William F. Finn, M.D., Professor of Medicine at the University of North Carolina School of Medicine in Chapel Hill, NC (UNCSM); and George Porter, M.D., Professor of Medicine and former Chairman of the Department of Medicine, Oregon Health Sciences University, Portland, OR (OHSU) and Sharon Anderson, M.D., acting Chairman of the Department of Medicine, OHSU. B.S. Kasinath, M.D. (University of Texas San Antonio Health Science Center) will participate in Phase I as a consultant. The radiology component of the study, including development of the final CT scan criteria and organization of the process of review by a Radiology Committee, will be directed by Richard Clark, M.D., F.A.C.R., (Professor and Director of GU Radiology at the University of North Carolina School of Medicine, Chapel Hill, NC). Dr. Clark will also be a Collaborating Investigator.

The Executive Committee for the study consists of Drs. Henrich and J. Shapiro; Drs. Kaufman and S. Shapiro; and from NIDDK, Lawrence Agodoa, M.D., Project Officer, and Diane Meeks, Contract Specialist. An External Advisory Committee, with responsibility for general oversight of the study, has also been appointed by NIDDK.

4. Summary of Phase I Activities

The major work in the initial phase will involve the following areas: final definitions of the subjects to be included; development of the logistical structure and methods for identifying and enrolling cases and controls; development of the CT scan criteria and the process for using the CT scan in the study; questionnaire design and field testing; and methods of communication, including transfer of interview data and CT scan results. Computerized logs for tracking patient enrollment and other relevant software for monitoring the progress of the study will be developed. Collaborative links between the coordinating center and the three regional clinical centers that will participate in Phase II will be established. To facilitate the development process, two categories of subjects will be enrolled during Phase I. The CT scan will be normalized in 60 subjects with normal renal function in North Carolina (30 each from UNCSM and WFUSM). Both the questionnaire and CT scan will be field tested on 50 ESRD patients (25 each from North Carolina and Oregon).

5. Timetable

The timetable for Phase I is shown in the attached figure. The formal start date of the project was October 5, 1999. Three meetings of the Executive Committee and two of the study investigators are allowed for, along with one meeting of the External Advisory Committee (EAC), and three meetings of the study Radiology Committee. The initial Executive Committee meeting was held on November 10; the first investigator meeting, in conjunction with a second Executive Committee meeting, was held on January 10, 2000, and the External Advisory Committee met the next day. Enrollment of the normal subjects for CT scanning began in the

second half of January, and will proceed through June. After the computerized interview and protocol for Phase I are completed, interviewer training is scheduled for late March/early April, with the enrollment of ESRD patients for interview and CT scan to begin immediately thereafter and continue until approximately the end of September. The Radiology Committee will meet to review interim data from the normal subjects in early April; the first meeting to review ESRD patients will be in June or July. Final evaluation of CT scans in the ESRD patients by the Radiology Committee will take place in October, followed by an Executive Committee and Investigator meeting. Delivery of the protocol for Phase II to NIDDK is planned for the beginning of November. That will allow a month for final discussions and decisions about Phase II before it commences in December 2000.

6. Progress in Administrative and Study Design Issues at the Individual Centers

A research nurse has been hired at each of the three Phase I study centers (WFUSM, UNCSM, and OHSU); and a laptop computer has been purchased at each center for data entry. Dr. Clark has spoken with members of the radiology departments at WFUSM and OHSU in order to discuss issues of CT machine compatibility. It will be optimum if the data from those institutions can be transferred digitally to the Radiology Committee for their interpretation of the findings, and this has been recommended by the EAC. Dr. Clark has also held discussions with the other members of the radiology team at UNC, who also are abdominal radiologists and have special expertise in abdominal CT. They are Dr. David Warshauer, Associate Professor, and Dr. Wendelin Hayes, Clinical Associate Professor. They will interpret the CT scans together with Dr. Clark. The radiology departmental chair at UNC, Dr. Joseph K.T. Lee, will serve as a consultant.

A major focus of the SEU during Phase I is development of the interview and computer database. Final input to the questionnaire design was obtained from all of the investigators at the January 10 Investigator meeting. It was decided to add a few questions regarding pain syndrome before the history of medication use is obtained, and to make a few additions to the list of indications for obtaining analgesic use. In conjunction with the local investigators, the SEU is developing a list of analgesic trade names and associated pictures that will be used to maximize subjects' recall. The current questionnaire (see Appendix 2), described below, is a modification of that submitted with the proposal. Members of the SEU staff have completed development of the computerized database system for the project. The interview questionnaire and the form for entering CT scan findings have been programmed in Microsoft Access. A manual of written instructions for administering the interview has been prepared (see Appendix 3).

7. Study Network

Subjects will be enrolled at three clinical centers, WFUSM and UNCSM in North Carolina, and OHSU in Oregon. ESRD patients will be recruited at all three centers; normal subjects for the CT scan will be enrolled only at the two North Carolina sites.

New ESRD patients at WFUSM will be recruited primarily from seven dialysis units owned by WFUSM. These units draw patients from a six county area within a 60 mile radius of Winston Salem (Forsyth, Guilford, Davidson, Iredell, Surry, and Stokes). Two of these units are located in Forsyth County, two in Guilford County, and one each in Surry, Davidson, and Iredell Counties. Only three dialysis units in these six counties are not owned by WFUSM, all in Guilford, but investigators at WFUSM have a history of successful collaboration with the group of nephrologists who use all three. By recruiting patients from all ten units, the study will have

access to essentially all new dialysis patients from a population base of approximately 1,023,570. As explained in the proposal (Section 5.1.2), there should be ample ESRD patients who meet the study criteria to reach the numerical goals of Phases I and II. Normal CT subjects will also be enrolled at WFUSM.

At UNCSM, new ESRD patients will be recruited primarily from four dialysis units operated by UNC, three units operated by Durham Dialysis in Durham, and two units in Raleigh, operated by Capital Nephrology and Wake Nephrology. These units cover Orange, Durham, and Wake counties (the so-called "Research Triangle"), and the contiguous counties of Chatham and Alamance. The combined population exceeds one million, which should provide sufficient patients to meet the study goals, as explained in detail in the proposal (Section 5.1.1). Normal subjects for CT scan will be enrolled at UNCSM in addition to the ESRD patients.

In Oregon, ESRD patients will be recruited from the Pacific Northwest Renal Services, which includes a network of ten dialysis units covering the metropolitan Portland and Vancouver areas. These units provide care for 57% of all hemodialysis patients in the area, which should be sufficient to meet the goals of Phases I and II (see proposal, Section 5.1.3).

8. Subject Enrollment

8.1 Normal Subjects

Enrollment of 60 individuals with normal renal function and urinalysis—10 individuals of each gender in three age ranges (35-49; 50-64; and $\geq$65)—has begun in North Carolina. Thirty volunteers are being recruited at each of the local centers (WFUSM and UNCSM). It was decided at the Investigator Meeting at NIH on January 10 that, given the higher incidence of ESRD among African Americans, one third of subjects in each stratum should be African

American. Exclusion criteria include: diabetes, nephrectomy, single kidney, current pregnancy, urinary tract infection during the preceding year, or abnormal urinalysis, creatinine, or electrolytes (as defined by the participating institutions).

Consent forms have been developed in accordance with the requirements of the IRB of each institution. There is an explicit statement that both the subject and his primary care physician will be notified of any abnormal findings detected by the CT scan,.

8.2 ESRD Patients

In early April, enrollment of the 50 subjects with ESRD will begin. The case population will be male and female patients with ESRD who have begun hemodialysis not more than two months previously. Data will be obtained at the local study centers by CT scan and interview. Patients with acute renal failure will not be included; other exclusions will be patients with diabetes requiring treatment with insulin or oral antidiabetics, polycystic kidney disease, renal transplant failure, biopsy-proven glomerulonephritis, amyloidosis, multiple myeloma, angiographically-proven renal artery stenosis, hereditary nephritis, AIDS nephropathy, acute renal failure that fails to recover, sickle cell disease, and toxic disease secondary to antineoplastic agents. These factors, when present, are overwhelming causes of ESRD. Patients who have had a nephrectomy will also be excluded. There will be a lower age limit of 35 years, because younger patients are unlikely to have used analgesics heavily enough over a long enough period to result in AN. Pregnant women will not be eligible because of the risks of the CT scan to the fetus. To ensure that such women are not inadvertently included, all women under the age of 55 years who deny being pregnant will be given a pregnancy test before the CT scan is conducted. Finally, eligible patients will be confined to those who reside within defined

catchment areas of the partcipating clinics; the latter restriction is necessary for the valid estimation of incidence, and it will also increase the feasibility of obtaining appropriate controls. Enrollment is projected to continue for six months, until approximately the end of September, 2000.

Details of the procedures for identifying ESRD patients that meet study criteria will be finalized during Phase I. The basic method of patient enrollment will involve the study nurses visiting each dialysis clinic in their regions at intervals of not more than every other week. Logbooks in the clinics will be consulted to identify patients who have begun dialysis since the previous visit. Whether or not the initial criteria are met in terms of age and the reasons for exclusion will be determined from the individual medical records; patients who potentially meet the criteria will be approached to participate in the study, and informed consent will be obtained (again, the consent forms have been designed according to the IRB requirements of each institution). The specific procedures for examining patient logs, obtaining medical records, obtaining informed consent, and scheduling CT scans and in-person interviews will be developed to fit in with the routine work in each of the dialysis clinics so as to cause minimal disruption.

9. CT Scans

9.1 Normalization

The normal subject scans will provide confirmation of renal dimensions in the general population and will supplement measurement data obtained by retrospectively reviewing 100 non-contrasted CT scans performed on patients with no obvious renal parenchymal disease. The latter activity is almost completed. The data will also be compared to published data on kidney

size and volume in preparation for providing the reference information against which the ESRD population will be measured. This needs to be done because there are few studies of normal kidney size published in the CT literature (most have used sonography or excretory urography) and none in which spiral CT techniques have been applied.

9.2 Methods of CT Scan Reading

For purposes of standardization, all CT scans obtained on patients from the regional centers will be read in the Department of Radiology at the University of North Carolina. Three experienced GU/CT radiologists will participate as "expert readers". They will interpret the CT scans that will be submitted in a uniform format (see below). During Phase I, before "official" readings commence, each of the three radiologists will interpret a series of test cases to determine his or her consistency and interpretative accuracy. It is acknowledged that not every radiologist reads examinations in an identical manner, and thus variations in opinions among the individual readers will be discussed as a group. Once it is determined that all three radiologists are familiar with the observation parameters (see below), interpretation of clinical cases enrolled for the study from the different centers will begin. *The radiologists will have no knowledge of the clinical status or analgesic history of any of the patients whose studies are submitted.* All measurements and scores will be agreed on by the three readers, and their final collective interpretation will be "the official interpretation" of whether AN is present or not.

9.3 Technical Issues of CT Scanning

It is likely that each of the participating institutions will have different CT scanners with different technical capabilities. Although it would be ideal if the interpretive methodology could

be applied to any type of non-contrast renal CT, a more rigidly controlled set of technical parameters is suggested for at least the initial phase of the study.

9.3.1 Type of Scanner

Because of the advantage of a true volumetric data set, we propose that scans be obtained with the "spiral" (helical) technique; each scanner must be capable of producing excellent images with the recommended standardized protocols. A local radiologist and CT technologist supervisor will be identified at each institution to help facilitate on-going image quality.

9.3.2 Patient Preparation

No patient preparation was specified in Elseviers' and De Broe's work[1] and none will be required for this study.

9.3.3 Scan Parameters

A single breath-hold spiral sequence through the kidneys is recommended with a slice thickness of 5 mm, pitch of 1 and reconstruction interval of 5mm. This should produce approximately 20-35 images per case depending upon renal size and position. Images will be obtained from l cm above the top of the higher kidney to 1 cm below the bottom of the lower kidney. Some parameters regarding the scan, such as KVP, MAS, field of view, and reconstruction algorithm, are of uncertain significance and will require some initial experimentation to establish their importance. Interpolating from the only prior work in this area (on calcified pulmonary nodules), it appears that lower KVP, smaller field of view and high resolution reconstruction algorithm would be the preferred technique, although how much

difference this will make over standard CT parameters is uncertain at this time. Initially, however, we will choose an MAS of 210, a KVP of 120, a standard soft tissue algorithm, and a 35 cm field of view (depending on patient size).

9.3.4 Display Parameters

For consistency, we will print the images utilizing a 12 on 1 format (14" by 17" film). Two sets of images will be printed. The first will utilize a standard soft tissue window width of approximately 250 HU with a center of 30 HU. The second set will utilize a narrow window of 2 HU with the level set at the minimum value acceptable for diagnosing calcification. This latter level will be in the 125-150 HU range.

Measurements of parenchymal thickness (specified below) will be made off the local workstation by the CT technologist and printed with the images of the case. This will greatly facilitate the interpretation of the case by the central Radiology Committee. It is acknowledged that the raw digital data from each scan would be most desirable to review centrally but differing output formats from each local CT scanner may preclude this.

9.4 Radiological Data to Be Analyzed from Non-Contrasted CT Scans

We will look at De Broe's criteria (renal "volume"; cortical indentations; and presence of papillary calcification,[1] because of their potential usefulness and their lack of validation by others. We will also employ more sophisticated radiological parameters to assess renal size, contour and calcifications, as described below. The final details of the criteria for AN will be determined during Phase I, based on our experience with the 60 subjects with normal renal function and the 50 ESRD patients.

9.4.1 Renal Volume

In De Broe's work,[1] renal "volume" was taken as an arbitrary number defined as the sum of width and height at the level of the renal hila. We favor a methodology based on CT scan that takes into account true renal volume rather than a sum of two renal dimensions taken from a single central slice. Although it will have to be normalized in our preliminary study, we favor a modified prolate ellipse formula as discussed by Hricak, whereby renal volume in cm^3 is equal to 0.49×length×width×AP dimension.[2]

Volume could then either be treated as an unmodified continuous variable, normalized for age, sex and body height, or treated as a categorical variable based on a cutoff value to be determined. Although the measurements needed to establish renal volume are more extensive than those used in the work of De Broe, these data could easily be obtained from each of the submitted scans either at the local institution or by the central reading facility. We believe that there is some advantage to expressing renal size as volume because it has greater relevance to autopsy data as well as to other publications in the radiological literature.

Another set of measurements that reflect only renal parenchymal thickness would be an alternative way of assessing renal size, perhaps with greater clinical relevance in patients with AN because of the frequent overall reduction in renal parenchymal thickness and its replacement by increased amounts of peripelvic fat. Assessing parenchymal thickness as a measure of renal size also could eliminate some of the problems encountered by alterations of renal position or rotation. Thickness of the renal parenchyma can easily be obtained by measuring parenchymal thickness on three contiguous hila slices and averaging each of the three parenchymal

dimensions (anterior thickness, posterior thickness and lateral thickness) (see proposal, Figures 12 and 13). This is a modification of a recently published technique.[3]

It may be that the assessment of renal size as reported by De Broe will be just as helpful in discriminating patients with AN as the more complex methodologies suggested above. However, U.S. radiologists should be more comfortable with the techniques described above because they reflect true renal size (length and volume), which also has more clinical relevance. Renal length as determined from urography, ultrasound, and CT is the most commonly utilized and accepted measure of renal size. Based on a review of the literature, and from discussions with fellow uroradiologists, the renal volume determination is not used as frequently as renal length. The evaluation of parenchymal thickness has received even less attention in the literature and in daily clinical practice. However, assessing the two latter parameters will be a beneficial byproduct of this investigation.

To summarize, a number of potentially useful measurements reflecting renal size and volume have been proposed. Data will be obtained to allow the evaluation of each of them; the parameter that ultimately proves to provide the best combination of discrimination between AN and other ESRD, along with wide applicability beyond the proposed study, will ultimately be employed.

9.4.2 Indentations

De Broe treated indentations categorically, classifying them according to the single level that appeared to contain the most scars. We will count all the indentations on the renal surface as recorded on the series of CT slices and treat this as a continuous variable. Inaccuracies due to the presence of normal fetal lobations will be taken into account. Distinguishing fetal lobations

from parenchymal scars due to AN or some other renal insult is often not straightforward. However, the indentations from fetal lobations usually are not associated with underlying parenchymal thinning. To assist in making the distinction, indentations will be classified as "minor" (<5mm) and "major" (≥5mm).

The assessment of cortical irregularity as performed by De Broe seems to be the least controversial of his parameters, except for the problems raised by the presence of fetal 1obations. However, there are many causes of renal scarring beside papillary necrosis (reflux nephropathy, infarcts, trauma, focal pyelonephritis). The ability to distinguish the causes of many cortical irregularities probably will not be possible from radiological findings alone.

9.4.3 Calcifications

De Broe also treats the presence of calcifications on the papillary line as a categorical variable.[4] What HU of CT density constitutes a calcification was not defined. We will define calcification to mean an HU density greater than a specific number between 125-150, to be determined, and recommend that the total number of papillae with calcifications be counted. From De Broe's work it is apparent that any papillary calcification suggests underlying AN with a relatively high sensitivity and specificity. As mentioned above, renal papillary calcifications or calcifications immediately adjacent to the renal papillae are likely to occur in many individuals not suffering from AN (patients with metabolic stone disease). Thus, it appears that a grading system for both number of papillae with calcifications (Class) and size (Grade) of papillary calcifications will be useful. We will assign a Class of 1 to kidneys with 1-3 papillae with calcifications, a Class of 2 to kidneys with 4-10 papillae with calcifications, and a Class of 3 to kidneys with greater than 10 papillae with calcifications. With regard to size of calcifications,

Grade 1 will indicate that the largest papillary calcification is less than approximately 2 mm; Grade 2 will indicate that the largest papillary calcification is less than 5 mm and Grade 3 will be assigned to kidneys with papillary stones greater than 5 mm in maximum diameter. We understand that different window and level settings may affect the apparent size of any renal calculi,[5] but some estimate is important together with their location (either in the papilla or in the adjacent calyx).

9.4.4 Other Observations

Each CT scan will also be evaluated for other findings of potential importance. For example, calcifications in locations other than the papilla (cortex, calyx, outer medulla, vascular) will be recorded. Cysts or other masses will be recorded and the amount of malrotation or other positioning abnormality of each kidney will be assessed because of its direct bearing on measurements of renal length and volume (see above).

9.5 CT Scan Diagnostic Criteria for AN

A major focus of Phase I will be development of the diagnostic criteria based on CT scan. The CT criteria must be precise enough to render a sensitive and specific diagnosis, and yet broad enough in the initial designation to include the possibility of detecting changes that may hold significance; the criteria must also yield reliable (i.e., reproducible) decisions from different reviewers. In previous studies, the specific diagnosis of AN has been made based on the demonstration of RPN in a setting of documented analgesic use.[6] However, this approach negates a fundamental principle of epidemiology: the fact of exposure *cannot* be used as a diagnostic criterion in a study undertaken to determine causation. To do so is to permit no

possibility other than a 100% exposure rate in the cases, regardless of whether analgesics do or do not cause AN.

9.6 Recording CT Information for Review

A data form, enclosed in Appendix 1, has been developed for recording the information from the CT scans and the assessment of the Radiology Committee. The form has been programmed in Access for direct entry of the information into the computerized data base. The evaluation of the CT scans will be conducted by the Radiology Committee in face to face meetings, with the participation of the Study Coordinator as a facilitator and recorder. This will help to ensure uniformity of the evaluations, and consistent, accurate recording of the information. Specifics of the information that will be recorded for each kidney are summarized below:

Initial information (page 1), including basic patient details and technical information concerning the scan.

Renal length and volume (page 2). Length will be calculated as the total number of slices in which renal tissue is seen×0.5 cm. Volume in cm^3 will be calculated as length×width×AP dimension×0.49.

Average parenchymal thickness (page 3) of three contiguous central slices, calculated as anterior+posterior+lateral÷3.

Kidney position and renal contour (page 4). Positional parameters to be recorded include malrotation, ectopia, and other aspects. For contour, the number of major (≥5 mm) and minor (<5 mm) indentations will be recorded.

Calcifications (page 5), including papillary calcifications, classified according to the number of papillae and graded according to size, and the location of any other calcifications.

Masses (page 6), including the number, size, and density, along with any extra-renal findings.

Decision of the reviewers (page 7). Subjects will be classified according to the De Broe criteria of decreased renal mass + bumpy contours + papillary calcifications, and according to new parameters for AN that will be developed during Phase I.

10. Interview

10.1 Questionnaire

The questionnaire has been designed to obtain valid information on analgesic use and relevant covariate factors (see Appendix 2). It has been programmed in Access, and the data will be entered directly into the computerized data base. The questionnaire will be the primary source of information that will be used to assess the performance of the CT scan. Particular attention has been paid to structuring the questions so that accurate and comparable information on regular analgesic use and the timing of early symptoms of renal disease (for cases) is obtained. This will allow the specification of exposure that occurred before the onset of ESRD. A summary of the information to be obtained follows. A detailed explanation of how to conduct the interview is contained in the study manual (Appendix 3).

Initial information (page 1), including age, sex, marital status, race, years of education, height, weight, and current diagnoses (obtained from the medical record).

Exclusion criteria (page 2), including diabetes, nephrectomy, and current pregnancy (for women under 55 years of age). The interview will be terminated if a subject answers

affirmatively to either of the latter two factors. ESRD patients who deny being pregnant will also be given a pregnancy test before undergoing the CT scan. Although diabetics will ultimately be excluded, the interview will be completed for those who respond positively to the questions about diabetes, because patients are known sometimes to have inaccurate information about that disease. The diabetic status of the latter individuals will be confirmed by the local investigator, who will then decide about exclusion.

Renal history (pages 3-5). This section of the questionnaire is of major importance, since the information will be used to time the onset of renal problems. The date of a first diagnosis of renal disease will be recorded, along with the date of first dialysis. Questions will be asked on conditions diagnosed by a physician that indicate renal disease (e.g., proteinuria, hematuria), specific renal diagnoses, kidney stones, urinary tract and kidney infections, renal x-rays, diagnoses of renal failure, and symptoms that are specific for renal disease. Hypertension will also be enquired about. Information will be obtained on the use of antihypertensive drugs (specific medications will be recorded).

Other medical history (pages 6-7). Information on various other relevant medical conditions will be elicited from all subjects, including gout, peptic ulcer, and cancer. The patient will be asked to list any other major or chronic illnesses, not covered in the specific queries. In addition, the patient will be asked about chronic pain conditions that normally lead to the regular use of analgesics, including frequent headaches, arthritis, and other pain conditions. Finally, the patient will be asked if there was a period when they had trouble getting through the day or difficulty sleeping. The questions on pain and the latter conditions are being asked separately from the drug enquiry in order to identify subjects who might not be reporting heavy analgesic use truthfully.

Analgesic history (pages 8-11). A lifetime history of regular analgesic use (defined as use at least once a week for at least three months) will be elicited by asking systematically about indications for use. The latter will include (prompt #1): pain, headache, backache, toothache, menstrual pain, muscle relaxant/spasms, arthritis, gout/high uric acid, swelling/inflammation due to injury, fever, cough/cold, and influenza. To capture prophylactic use, analgesic abuse, and use due to vague symptoms of renal disease, the list will also include the following indications (prompt #2): "help sleep," "get through or manage the day," "to reduce stress", "perk up," "not feeling well," to treat or prevent "hangover", prevent headaches or other pain, prevent heart disease, prevent thrombosis, and prevent "restless" legs. The indications will be followed by a list of the trade names of commonly used products in all analgesic categories, i.e., aspirin, acetaminophen, combination products, NSAIDs (prompt #3). The current version of the list is included with the questionnaire (page 9); it will be finalized during Phase I. To further assist recall, subjects will be shown a book with pictures of analgesic products used in the U.S. in the past 20 years. The book is presently under development. Finally, subjects will be asked about any other pain medications not covered by the specific indications or trade names (prompt #4).

Drug names will be coded based on the SEU drug dictionary which cross links trade and generic names of drug products with their components. This sophisticated and powerful tool was developed in 1975 by the SEU research pharmacist and has been continuously updated. It allows the evaluation of individual products (e.g., Tylenol), or groups of drugs containing the same active ingredient (e.g., all acetaminophen-containing products).

The reference point for enquiring about drug use (as well as most other information) will be the date of initiation of dialysis, which will provide a standard and recent event that the interviewers and subjects can focus on (for controls, the date of interview will be the reference

point). The details of each episode of regular use before this point will be recorded, including year started, duration, frequency of use, number of pills per day, and specific reason for use. It would not be possible to obtain a reliable lifetime history of occasional or very short term analgesic use by interview, and we will not attempt to do so. That will not be a problem, however, because the hypotheses are concerned with regular long term use, which can be reliably obtained by the approach set forth here.

It will not be feasible to obtain explicitly the milligram dose of each product used over an entire lifetime. However, the doses are fixed in combination products (although these may vary over time), and in single ingredient analgesics are usually inherent in the product name, which will be recorded. The use of pictures will be particularly helpful in obtaining the precise names. Thus, in many instances it will be possible with the names, dates of use, and number of pills per day to infer the daily dose, and hence the total lifetime dose. Of course, it will not always be possible for the subjects to remember the specific trade names, in which case the dose values will be considered to be unknown. With the use of pictures, however, we judge it likely that when the names are reported, they will be reliable.

An additional hypothesis concerning analgesics and renal disease is that use of the drugs exacerbates the progression of renal insufficiency to ESRD. In the proposal (Section 2.1), we concluded that this hypothesis could not be definitively evaluated in an observational study, because of the likelihood that the choice of analgesics is influenced by pre-existing renal disease (confounding by indication). However, some insights can be gained by obtaining information on changes in analgesic use after the first diagnosis of renal disease: to this end, patients will be asked whether a physician advised them to change or discontinue use of pain medications

because of problems with their kidneys. Those who respond affirmatively will be asked whether they stopped or switched the medication.

Family history (page 12), including diabetes, hypertension, and ESRD.

Habits (pages 13-14) of consumption of tobacco, coffee, tea, and alcohol. Beverage consumption at age 30 will be recorded, as well as current consumption (i.e., before starting dialysis).

Occupational history (page 15), including a brief description of each job held for over one year, the year it was started, and its duration.

Income (page 16). This information is placed late in the interview because of its sensitive nature.

Miscellaneous information (page 17). Details of the use of illicit substances will be enquired about, with an explicit statement about the confidentiality of the response. Because this information is the most sensitive, and hence the questions about it have the greatest potential to alienate study subjects, they are placed last so as not to jeopardize the rest of the interview.

The questionnaire will be administered to all study subjects in person. Interviewers will use a specially designed book of pictures of analgesics to aid in recall of medication use, thereby maximizing the completeness of the information. Routine quality control procedures will include detailed review of each interview by the Study Coordinator in Boston, automatic computerized checking of the data for consistency, and prompt correction of errors that are discovered, with feedback to the interviewers on an ongoing basis.

Considerable attention will be paid to training the interviewers in the clinical centers so that the information obtained will be standardized and comparable. The training will be conducted at the local sites by the SEU staff, and will take place at the end of March 2000. All

study methods and procedures will be covered, including developing the mechanisms for identifying potential cases, obtaining information on possible exclusions, obtaining informed consent, scheduling the CT scan and the interview, maintaining a log of study subjects, including reasons for nonparticipation, and administering the interview.

10.2 Validation of the Questionnaire

It is always difficult to validate interview information, because there is seldom an absolutely reliable "gold standard" against which the questionnaire data can be measured. While that situation applies in the present instance, an indirect approach to validation will be used during Phase I to gain insight into the accuracy of the histories.

Twenty ESRD patients interviewed during Phase I will be selected at random and reinterviewed at least three months after the initial contact. We will then conduct a detailed comparison of each individual's responses on the two questionnaires. If the responses are similar, it will suggest that the questionnaire elicits reproducible answers. If the two sets of interviews do not yield similar results, we will redesign the questionnaire as needed before embarking on Phase II, guided by the characteristics of the observed differences.

11. Data Management

11.1 Quality Control

Attention to quality control of the data will be a major emphasis of the SEU in its role as Coordinating Center of the current project. In addition to on site training of the interviewers, the following procedures will be employed.

Automatic consistency checks of the data have been built in to the computerized interview. Some examples of automatic error checking and rejection of inappropriate values include: providing predefined choices for categorical variables with the use of drop-down lists; warnings when questions are not answered; warnings on outlying values (e.g., more than 50 alcoholic drinks a day); checking for valid dates; checking for valid ages (e.g, the age at diagnosis of a medical condition cannot exceed the current age); checking that the year analgesic use started is consistent with the duration of use (e.g., the year started plus duration must not be later than the current year); automatic skipping of questions that should only be answered if there is an affirmative answer to a previous question; online coding of drugs with the SEU drug dictionary, which will prevent misspelled drug names and coding errors.

Each interview will be individually reviewed by the Study Coordinator in Boston for completeness and consistency; errors will be corrected promptly, in collaboration with the local interviewer as necessary. The Study Coordinator will maintain regular telephone and e-mail communication with the interviewers to ascertain problems, clarify questions about the data, and provide feedback on performance. During Phase II, a site visit will be conducted to each of the two regions by the Study Coordinator and Co-Investigator or Epidemiologist. There will also be one meeting of the data collection staff in Boston during Phase II; the latter is important to assure the consistency and comparability of the data. In addition to the ongoing quality control activities, the first step in analyzing the data will be for the epidemiologist to check the data for outlying values and computing errors. Any errors in the raw data that are discovered during this process will be corrected.

Quality of the radiologic data will be ensured by the use of a rigorous and standard protocol for conducting and printing the scans, as described above. Relevant data from the

scans, along with the assessment of the Radiology Committee, will be recorded on standard forms during the review sessions. To maximize the consistency of that process, the Study Coordinator will participate in the meetings as a recorder and facilitator.

11.2 Data Handling Procedures

Secure master data files will be maintained at the Coordinating Center in Boston. To assure confidentiality, individual subject identifiers will not be included in the data files. The records will be identified only with an anonymous study number. If individual identifiers are required (e.g., if it is necessary to contact the patient concerning results of the CT scan), these will be kept in separate manual files in the regional study centers, that cannot be linked to the master files by computer.

Data will be electronically transmitted to the SEU every week over a virtual private network (VPN) with an enhanced firewall; the VPN allows a connection to the Unit's computer system through a local internet service provider or academic computing center with internet access while still maintaining security. An automatic upload procedure developed by the SEU computing staff will transmit an encrypted database containing the interviews that have not previously been sent. These will then be added to the master data file for quality control. At regular intervals, a copy of the quality controlled data will be transmitted back to each center. Previously uncorrected interviews on the local database will be automatically replaced with the "clean" data, so that each center will have a copy of the interviews conducted at that site that is as up to date as possible.

Interview data will be backed up daily onto Iomega Zipdrives, using a procedure developed by the SEU computing staff. Since each interview computer will contain a copy of

the Access database, it will be straightforward to simply copy it from the hard drive to the Zipdrive. Three backup copies will be maintained on a rotating basis, with each copy being overwritten every third day. The main database on the SEU server will be backed up daily to a tape drive. All data on the servers are automatically backed up in this way, with procedures that run automatically during the night. Server backups are maintained on a rotating basis for seven days. Friday night backups are rotated offsite weekly, and rotated back in four weeks, with the exception of the backup from the first Friday of the month, which is permanently archived offsite. Analytical data files will also be kept on the server, and thus automatically backed up.

An audit trail will be maintained, using a number of steps. First, data transmitted from the local centers will be copied to the active database, and also to an inactive copy to which no changes will be made. The latter will provide a record of the data as originally collected; the interviewers will be identified, and an automatic date and time stamp for each interview will be included. As part of the quality control procedure, the Study Coordinator or any other person making changes to the data (e.g., the Epidemiologist) will first log their name in to a quality control startup dialog in the main active database. Any changes that are made during that session will then automatically be recorded in a table which will contain the subject ID number (an internal study number to which all data for that individual are linked), field changed, old value, new value, and the date, time, and name of the person making the change. The procedures will provide a comprehensive archive of the original raw data and a change log where all differences between the original and working dataset are specifically identified. These measures will allow the reconstruction of the sequence of events for any and all changes made, with considerable ease and flexibility (e.g., the log could be sorted by ID number, date, person making the change, field changed, or some combination of factors). A particular advantage of

the automatic logging of changes is that it will be minimally burdensome to study personnel in the ongoing performance of their regular duties; it will therefore be not only efficient, but also cost-effective.

12. Data Analysis

Since the objective of Phase I is development of the study procedures, it is not anticipated that substantive results will be forthcoming, nor will there be enough enrolled subjects to allow for detailed anayses. However, we plan to conduct simple analyses as part of the evaluation of the quality of the data, and to help determine whether changes should be made to the interview. These will include frequency distributions of all the variables on which information is obtained, which will help to determine the usefulness of the individual questions. As already noted, there will also be a detailed comparison of data from the "before" and "after" interviews of the 20 reinterviewed patients to check for consistency. In addition, the 50 ESRD patients will be classified according to their use of various analgesic drugs. Users of a given drug or group of drugs (e.g., Excedrin, all acetaminophen-containing products) will be divided into regular (e.g., ≥4 days a week) and less frequent users, and further subdivided according to duration of use (e.g., <5, ≥5 years). An index day corresponding to the earliest renal symptoms or diagnoses will be specified, and use that occurred before a predetermined interval preceding the index day (e.g., two years), will be examined. This approach, which we have termed "lagged exposure analysis," is discussed in detail in the proposal (Section 5.3.2). The ESRD patients will be further classified according to AN as determined by the De Broe and new criteria, and crude correlations with analgesic use defined as above will be evaluated. During Phase II, multivariate analysis will be employed to control for confounding by covariate factors; there will not be

sufficient data for this purpose in Phase I, nor even for stable crude analyses. Thus, although some preliminary information may be provided on the direction of the correlation of the CT criteria with analgesic use, those findings will mainly be useful for planning modifications to the protocol for Phase II, or for justifying the existing procedures.

A more detailed description of analytical procedures will be provided in the protocol for Phase II, since it is during that phase that substantive results will be generated.

13. Study Design Issues

In this section, a summary of the methdological issues that have guided the study design is provided.

13.1 Selection Bias

In an unbiased study, the reason for selecting any subject should be independent of the exposure. If it is not independent, there is selection bias. In the current study, we will avoid such selection bias by obtaining exposure information subsequent to identification for the study.

Selection bias may also arise if a large proportion of eligible subjects targeted for inclusion are not successfully enrolled, and if the rates of analgesic use differ between the subjects who are enrolled and those who are not. In the context of AN, selection bias could arise if otherwise occult uremia were selectively diagnosed among analgesic users (e.g., if they are under active medical care because of arthritis, and thus more likely to have diagnostic tests performed). Similarly, bias could arise if there was a selective tendency to perform CT scans on uremic patients (and thus to identify features compatible with a diagnosis of AN) if they had used analgesics, but not otherwise.

In this study, selection bias will be minimized by identifying *all* ESRD subjects in the participating centers who meet the definition criteria of the study and enrolling as many as possible. A number of approaches will be taken to minimize losses. The requirement that patients be identified within 30 days of the commencement of dialysis will reduce the number that cannot be located, or cannot be interviewed because they are too sick. It will also ensure that few have yet begun home dialysis, increasing the feasibility of conducting the interviews in the clinic. If possible, the interviews will be conducted while the patients are actually being dialyzed. That arrangement should further enhance participation, because the patients will be available for interview for a number of hours.

13.2 Information Bias

Information bias, or differential reporting of exposure histories by cases and controls, is a major methodologic concern in the current study. Because of general concerns in the medical community about AN, patients with otherwise unexplained renal failure are likely to have been questioned carefully about their analgesic use by medical personnel in advance of their interview for the study. Controls will not have this prior stimulation of the recall of their analgesic use, and will probably be less motivated to probe their memories.

In the current study, stringent measures will be taken to ensure the completeness and comparability of the information from all subjects. We will use a highly structured questionnaire, designed to maximize the completeness of recall, and interviewers will be carefully trained to conduct it in the same manner with all subjects.

In the analysis, information bias will be minimized by placing the greatest reliance on evaluation of the most heavy analgesic use categories. For example, if there is a strong

association, between ESRD and daily use of a particular analgesic for five or more years, with the reference category being subjects who have never been exposed, we would judge the association to be valid.

13.3 Misclassification

In any causal research an absolute requirement for validity is that an exposure to an agent must antedate the onset of the disease it is alleged to cause. This requirement, although obvious, is difficult to satisfy because of the insidious and chronic nature of the changes that follow the onset of AN, and the impossibility of pinpointing exactly when renal insufficiency began. In addition, symptoms due to the underlying renal insufficiency (eg., fatigue, lassitude, nausea, and headaches) may "cause" analgesic use (confounding by indication), rather than the reverse. To evaluate the hypothesis that analgesics cause renal disease *de novo*, it is necessary to restrict the evaluation to analgesic use that took place before the onset of disease, and that date can seldom be established with precision.

As much information as possible will be obtained on symptoms and renal abnormalities (e.g., elevated creatinine level) that are indicators of renal insufficiency; the information will be used to specify an index date that will be the month and year of the earliest indicator. The index date will be an approximation, and it is acknowledged that for all subjects, the actual disease process will have begun earlier. To allow for this, lagged exposure analysis will be used: all exposures in a defined period prior to the index date, such as one year, will be censored. This procedure creates an "effective index date" that is one or more years earlier than the presumed index date. In Phase II, an index date will be specified for each control that is equivalent to the date for the matched case, and exposure will then be defined in the same manner.

Despite the uncertainty about the onset of renal disease, it is reasonable to assume that regular analgesic use that lasted for many years before the best estimate of disease onset would have commenced before the illness began. It will not be possible to evaluate short term or occasional analgesic use in this study because reliable lifetime histories of such use cannot be obtained retrospectively by interview.

The potential for misclassification of outcome also exists. Inasmuch as the CT scan criteria are subjective, with room for variation in observer judgement, there could be some misclassification in the assignment of AN status. Such misclassification would reduce the precision of the CT scan as a diagnostic tool. However, as noted above, it will not be biased according to exposure status, since that information will not be known to the reviewers. The effect of individual variation will be minimized because the decisions will be made collectively by a three member Radiology Committee.

13.4 Confounding

Confounding arises when an apparent association (or lack thereof) is explained by one or more factors related both to the exposure and the outcome. The principle in controlling confounding in case-control studies is to compare cases and controls within similar categories of the confounding factor. In the present context, with the emphasis on analgesic use prior to the onset of renal insufficiency, the main confounding is likely to come from the use of multiple analgesics at different times or at the same time. Such confounding can be controlled by multivariate analytic techniques, since information on such exposure will be obtained, in addition to information on other factors that might influence both analgesic use and the likelihood of ESRD, such as socioeconomic status, race, and prior medical history.

14. Preparation for Phase II

As already explained, we plan during Phase II to enroll and interview one normal control for each ESRD patient, matched for race, decade of age, sex, and region. Although controls will not be included in Phase I, the most appropriate method of identification and enrollment will be determined during that time. Possibilities to be explored include random digit dialing (RDD), and drivers' license or social security records. RDD is well established as a valid approach. Although the increased use of answering machines, fax machines, and computer lines has decreased the efficiency of the method somewhat, it remains feasible. At present, we are actively using RDD in a national population-based survey of health behaviors. In the pilot phase of the survey, with approximately 1800 numbers called, 5% were fax or modem lines; we judge that most of those were secondary lines at residences or businesses used solely for those purposes, and thus not a source of eligible subjects. A further 4% were answering machines, and we were unable to speak with a person. We consider such a loss rate to be acceptable, and the actual proportion is probably lower, because not all of the answering machine numbers would have had an eligible subject.

Based on the above considerations, and since it is feasible in all study regions, we currently project RDD as the primary approach for recruitment of controls. Whether or not the other methods will be feasible in the study regions will be clarified by contacting motor vehicle registries and local social security offices, following which a final decision will be made.

Preliminary data from WFUSM and the Research Triangle area suggest that approximately 25% of incident ESRD patients in North Carolina would be eligible for the study. According to the USRDS data for 1996, approximately 49% of incident ESRD patients in

Network 6 (North Carolina, South Carolina and Georgia) had a non-analgesic related cause of ESRD. This implies that approximately 51% of incident cases would be suitable for the current study. The cause for this apparent discrepancy between USRDS data and the data obtained from our individual dialysis units will be determined during Phase I. We will also check the accuracy of diagnoses submitted on the Medicare form by surveying the original patient records on a sample population of patients starting dialysis.

15. Conclusion

It is anticipated that the 14 months of Phase I, which have been carefully planned, will allow for the full development of study procedures, and for the resolution of most methodological problems that are likely to arise during the conduct of NANS. If necessary, the protocol for the next phase of the project will be modified based on the experience during Phase I. The staged approach, which was mandated by the original RFP from NIDDK, should provide a reasonable assurance that the study can be completed successfully before the substantive work of Phase II is embarked upon.

16. References

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APPENDIX 1

RADIOLOGY DATA FORMS

APPENDIX 2

INTERVIEW QUESTIONNAIRE

(paper version)

APPENDIX 3

INTERVIEW INSTRUCTION MANUAL

(for computerized version)