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Looking for Trouble: The Added Value of Sequence Analysis in Finding Evidence for the Role of Physicians in Patients' Disclosure of Cues and Concerns

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ABSTRACT

Background: Not knowing patient concerns can lead to misunderstandings, incomplete diagnoses, patient dissatisfaction, and nonadherence. Although many studies show relations between physician communication and patients' expression of cues or concerns, most of these studies are cross-sectional, thus limiting the interpretation of these relationships. Sequence analysis can show the immediate effects of physician communication behaviors.

Objective: To show the added value of sequence analysis in finding evidence for the role of physician communication in patients' disclosure of cues and concerns.

Research Questions: Which physician communication predicts patients' expression of cues or concerns when using 2 different types of analysis: sequence analysis and cross-sectional analysis?

Methods: In a sample of 99 videotaped medical encounters with hypertensive patients in General Practice, we coded communication with Roter Interaction Analysis System and timed physician eye contact. For the cross-sectional analyses, we performed Poisson regression analyses to establish which physician communication is related to the total amount of patient cues and concerns. For the sequential analyses, we performed logistic regression analyses to establish which physician communication is directly followed by cues or concerns. We report incidence rate ratios and odds ratios (ORs), respectively.

Results: Both methods show that physicians' facilitative communication (1.21 and 2.33, respectively), eye contact (1.02 and 1.51, respectively), and psychosocial questions (2.42 and 3.50, respectively) are related to more disclosure of cues and concerns. Moreover, sequence

analysis shows that patients' expression of cues or concerns is less often preceded by physician social talk (OR = 0.49), giving instructions (OR = 0.38) and providing biomedical information (OR = 0.45) or counseling (OR = 0.39). In the cross-sectional analyses, these relations are absent or—before controlling for confounding variables—even in the opposite direction. All reported results are significant at $P < 0.01$ or $P < 0.001$.

Conclusions: Although cross-sectional analyses and sequence analyses show grossly the same results, sequence analysis is more precisely in demonstrating the direct influence of physician communication on subsequent cues and concerns by the patient. Physicians should avoid long monologues with medical information and should use facilitative communication, eye contact, and psychosocial questions to help patients express themselves.

Despite widespread beliefs that patients have become more equal partners in healthcare encounters, the scarce empirical studies that really looked into the dynamics of medical visits show a different, more complicated picture. In fact, most patients leave a medical encounter with unvoiced agendas.1 Patients desire information but make few attempts to elicit it from doctors.2 In most medical visits, patients ask surprisingly few questions,3–6 and this has not improved over the years.6 Usually, physicians are still running the show, determining the course of the dialogue, and using several strategies to control the medical visit.7

Social and emotional agendas are the most likely issues to be underrepresented in the medical visit.8,9 Although many patients enter the medical visit with emotions, these often remain implicit throughout the encounter, and only indirect cues indicate that something is worrying them.10–13 In 57% of General Practice encounters, patient do not present any concern.9 Both physician and patient are involved in this process.14 Physicians may discourage patients from disclosing details regarding their social and emotional issues by privileging the biomedical aspects of patient complaints.7,9 Patients, in turn, may be worried about what is deemed appropriate to communicate and about wasting doctors' time 1,15 and discuss their emotions only if the physician initiates the discussion.16 This could compromise the quality of care, because unvoiced patient concerns can lead to misunderstandings, incomplete diagnoses, dissatisfaction, and nonadherence.17–19

Over the years, several empirical studies have analyzed which types of physician communication facilitate patients to disclose their worries. In a comprehensive review, Zimmermann et al 9 found several of behaviors to be conducive to patients' disclosure of emotional information, including adopting a reassuring and empathetic interviewing style, listening to patients attentively, and targeted psychosocial questions. Biomedical information giving was positively related to patients disclosing concerns in some studies, but there was some debate about the causal direction of this relationship. Biomedical questions seemed be related to fewer patients concerns.9

However, all these studies were based on cross-sectional designs, impeding inferences about temporal relationships. Important questions such as: Do patients talk about concerns after their physician has displayed attentive listening or Do physicians listen attentively because their patients started talking about their worries? cannot be answered with this type of analysis. In cross-sectional designs, both interpretations can be valid. Consequently, several researchers have recently started to apply sequence analysis to their data, a method which originates from Conversation Analysis (CA). In this type of analysis, the order of events is analyzed, providing the opportunity to study how physicians and patients react to each other. Sequence analysis seems specifically suitable for studying rare events, which would otherwise get lost in the vast

amount of simple conversational acts relating to information exchange.20 In a review of 10 studies that used sequence analyses, the authors found that silence, facilitative communication, and open questions, especially with a psychosocial content, increased the chance that the patient subsequently expressed a cue or concern. Mixed results were found regarding the effect of medical information giving.9

At first glance, the results of the 2 approaches in the literature—the cross-sectional analyses of summative data and the sequence analysis of single utterances—seem to be very similar. This raises questions about the added value of sequence analysis above the more conventional statistical techniques. However, until now, these techniques were never applied in combination to the same dataset, which has left this question open to speculation.

Against this background the following research questions will be answered:

1. Which types of physician communication correlate with patients' expression of cues or concerns in summative statistics of medical encounters?
2. Which types of physician communication precede patients' expression of cues or concerns in sequence analyses?
3. What is the added value of sequence analysis over summative statistics in studying physicians' influence on patients' expression of cues or concerns?

On the basis of the literature, we expect patients to express more cues and concerns when physicians use affective communication, such as empathy and establish eye contact. With respect to task-oriented exchange, we expect that physician questions might invite patients to offer their cues or concerns, whereas information giving and counseling might steer patients toward listening rather than talking. Surveying the literature, we do not expect to find many differences in results between cross-sectional analyses and sequence analyses. However, even if there are no differences, sequence analysis could have added value, because it provides more support for the likelihood of a causal link between physician communication and patients' immediate reactions, as the order and contiguity of the events are taken into account.21

METHODS

Sample

The medical encounters in this study were drawn from a large body (n = 2784) of videotaped medical encounters from 142 Dutch general practitioners, recorded as part of the Second Dutch National General Practice Survey.6,22 Most physicians (75.8%) were men, and 66.7% were working in a group practice or health center. Medical encounters were videotaped by an unmanned camera. Overall 88.1% of the patients were included after giving written informed consent. To attain a homogeneous sample of medical encounters, only encounters with hypertensive patients were selected for this study.23 Hypertension is a common health problem requiring serious medical attention and a biopsychosocial approach and has a sufficiently high frequency to allow statistical analysis.24 A total of 108 GPs saw at least 1 hypertensive patient on the day of video recording. If there were more encounters with hypertensive patients, the first one was selected. Encounters with non-Dutch speaking patients and/or multiple persons were excluded, leaving 99 medical visits (from 99 GPs) for the analyses. Patients' mean age was 61.7 years (standard deviation [SD]: 14.03), and 64% was women, which is comparable with other studies on hypertensive patients in General Practice.6

Coding

Videos were coded with specialized observation software (The Observer, Noldus), and time and sequence stamps were automatically added during the coding procedure.

Verbal communication was coded with the Roter Interaction Analysis System.25 RIAS is widely used for assessing patient-health provider communication and has proven validity and reliability.26–28 In RIAS, all utterances are categorized in mutually exclusive categories that are the same for both patient and physician. RIAS categories were aggregated into meaningful categories, based on factor analysis and consistency with previous publications.6,29 RIAS coders were trained and supervised by the first author. Interrater reliability assessed with Pearson r coefficients was good, ranging from 0.72 to 0.99 for different categories.

Patient cues and concerns were coded using the consensus definition by the Verona Group for Sequence Analysis, VR-CODES (in press). VR-CODES defines a cue as "a verbal hint which suggests an underlying unpleasant emotion and would need clarification from the health provider" and a concern as "a clear and unambiguous expression of an unpleasant current or recent emotion where the emotion is explicitly verbalized." Interrater reliability was validated by independently double coding 10 medical encounters with different raters. The agreement between both raters was considered satisfactory (70% agreement). GPs' eye contact was continuously coded as patient-directed gaze.

Data Preparation

For the cross-sectional analyses, verbal communication codes were aggregated to frequencies per medical encounter. Patient participation was calculated as percentage of patient utterances on all utterances and eye contact as percentage of the total duration of the medical encounter.

For the sequential analyses, the RIAS codes (N = 22,764), cues and concerns, and eye contact were imported into SPSS 14.0 together with their time and sequence stamps. Patients' RIAS codes were tagged as cue or concern on the basis of time-proximity. All RIAS codes were also labeled as coinciding with patient-directed gaze or not and as being uttered by patient (10,970 utterances) or general practitioner (GP) (11,794 utterances). We used the SPSS lead function to identify whether variables of interest were directly followed by cues and concerns. For RIAS categories, we focus on the 2 physician utterances immediately preceding a patient cue or concern. The decision to use 2 preceding utterances was because including longer stretches of sequences do not seem to add much information.12,30 For patient participation and physician eye contact, the existing literature gives few indications about the optimal number of lags; hence, we used 5 preceding utterances.

Statistical Analysis

Basic statistical analyses were performed in SPSS 14.0 and regression analyses in MlwiN 2.02. For the cross-sectional analyses, frequencies of cues or concerns were used as the dependent variables in univariate Poisson regression models allowing for extra Poisson variance to account for over or under dispersion. We report incidence rate ratios (the exponentiated Poisson regression coefficients). For the sequential analyses, we used multilevel logistic regression models with a random intercept to control for clustering of communication sequences within medical encounters. The sequence is coded in a binary dependent variable: a physician utterance is either followed by a cue/concern (1) or not (0). We report odds ratios (ORs). To check whether communication sequences are sufficiently stationary throughout the medical encounter, we repeated all analyses using only the first cue or concern in the medical

encounter.

We repeated all analyses controlled for age and gender of both patient and GP (unreported), but this did not affect the results.

RESULTS

In 85% of the medical encounters, patients present at least one cue or concern. Cues are more common than concerns. Patients express, on average, 2.44 (SD = 2.34) cues and 0.77 (SD = 1.39) concerns per medical encounter. The mean frequency per medical encounter of cues and concerns combined is 3.21 (SD = 3.18).

Cross-Sectional Analyses

Cross-sectional regression analyses show (Table 1) that patients express more cues and/or concerns in medical encounters where the physician provides substantial amounts of medical information, talks about psychosocial issues, and frequently uses facilitative communication (empathy, paraphrasing, showing agreement, and backchannel responses). More eye contact is associated with more cues or concerns. Patients who are overall more active as indicated by the percentage patient participation are likely to express more cues or concerns. In general, the same patterns are found for cues and concerns.

[TABLE 1]

Sequential Analyses

Tables 2 and 3 present the results from the sequential analyses. More often than other types of patient utterances, a patient cue or concern is embedded in a longer stretch of patient talk. If a patient is not interrupted for at least 5 utterances, the probability of a cue or concern is almost 6 times higher than immediately after a physician utterance (Table 2). GPs' eye contact increases the probability of a patient cue or concern: if eye contact is present during the 5 utterances preceding and during patient's talk, the OR for a cue or concern is 1.51 ($P = 0.004$, not in Table 2). The same patterns are found when looking only at the first cue or concern per medical encounter. Table 3 shows that the odds of a patient expressing a cue or concern are almost 2-fold higher after GPs affect-oriented communication (OR = 1.97, $P < 0.001$) but lower after GPs' task-oriented (OR = 0.67, $P < 0.001$) or process-oriented communication (OR = 0.47, $P < 0.001$). In more detail, patients are more likely to offer a cue and/or concern after physicians' facilitative communication (OR = 2.33, $P < 0.001$) or psychosocial questions (OR = 3.50, $P < 0.001$) but less likely to do so after GPs' social talk (OR = 0.49, $P = 0.001$), instructions (OR = 0.38, $P < 0.001$), biomedical information giving (OR = 0.45, $P < 0.001$), and counseling (OR = 0.39, $P = 0.001$). Again the same patterns are found for cues and concerns. Repeating the analyses for only the first cue or concern in the medical encounter yielded roughly the same results (not in Table 3).

Comparing the predictor variables of cues/concerns in cross-sectional analyses and in sequence analyses, generally the same pattern is found: positive relationships are found for patient participation, eye contact, psychosocial questions, and facilitation. No relationship is found for biomedical questions, psychosocial counseling, lifestyle talk, concern or optimism, seeking dialogue, and disagreements. There are some discrepancies between the 2 types of analyses:

* Biomedical information giving shows a positive relation with the number of cues and concerns (cross-sectional analyses) but a negative relationship with the probability of a subsequent cue or concern (sequential analyses).

* For social talk, process-oriented talk, and biomedical counseling, there is no correlation with the total number of cues/concerns (cross-sectional analyses) but a negative relationship with the probability of a cue or concern directly afterward (sequential analyses).

* For psychosocial information giving, a positive relationship with the total number of cues/concerns is found (cross-sectional analyses), but directly afterward there is no change in probability of patients expressing a cue/concern (sequential analyses).

[TABLE 2]

[TABLE 3]

DISCUSSION

Nearly 25 years ago, Inui and Carter 31 accused researchers in doctor communication of reducing the medical encounter to a flat bundle of statistics by only presenting frequencies and calculating correlations, thus ignoring the interactions between the 2 main players: the doctor and the patient. It was—they argued in Medical Care—as if Hamlet would be described as "a play with 21 principal characters, a ghost, a group of players, and various numbers of lords, ladies, officers, soldiers, sailors, messengers, and attendantsone of whom is already dead, – one of whom dies by drowning, one poisoned by drink, two by poisoned sword and one by poisoned sword and drink." A more vivid way to describe the lack of dynamics in doctor-communication studies would be hard to find. However, most quantitative researchers, including our own research group, continued to describe videotaped medical encounters based on frequencies of physician and patient communication behaviors per medical encounter and to analyze these data by calculating correlations between these frequencies. Only recently researchers started to use techniques that focus on the interaction between doctor and patient, for example, by analyzing turn taking 32 or applying sequence analysis to the coded data.13,23,33,34 This study is the first to combine the more conventional approaches with the technique of sequence analysis in the same dataset.

This combined approach shows some interesting results. First, it is important to note that throughout the study the same relationship patterns were found for cues and concerns, suggesting that cues and concerns may be different in the degree of explicitness of expressed emotion, but they seem to be evoked by the same type of GP communication.

Second, it is important to stress the similarities in results of both methodological approaches, ie, the cross-sectional approach and the sequential approach. The Rogerian 35 communication skills such as showing empathy, paraphrasing what the patient just said, and showing agreement with the patients' words by backchannel responses have a meaningful relationship with patients' hints to or expressions of concerns and worries. So does eye contact and asking questions in the psychosocial domain. The fact that this result is found in cross-sectional studies and in sequential studies implies that this is a very robust result. From a clinical point of view, this means that when GPs want to know more about their patient's worries or concerns, they should use these techniques in the medical dialogue.

However, apart from the similarities between the 2 approaches, it is also important to pay attention to the differences in results. Two issues deserve special attention. The first is the function of social talk within the medical encounter. In the cross-sectional analyses, there is no

relationship between social talk and patients' expression of cues or concerns. The sequential analyses even show a negative relationship, meaning that patients are less inclined to talk about their concerns immediately after GPs' small talk, which is usually meant to create a relaxed atmosphere for the medical visit. Earlier studies found a negative relationship between social talk and the amount of patient talk about psychosocial issues,36 which is consistent with this result. This means that joking or making personal remarks to the patient might have a relaxing function in the medical encounter (we cannot tell from this study), but contrary to the intuition of many doctors, it is certainly not enough to facilitate patients to start talking about their worries.24

The other remarkable difference between the cross-sectional analysis and the sequence analysis is the relationship between GPs' provision of biomedical information and patients' offering a cue or concern. In the cross-sectional analyses, we found a positive relationship between GPs' provision of biomedical information and patients' offering cues or concerns. Sequential analyses revealed a different finding: the odds of a patient providing a cue or concern were substantially lower immediately following GPs' provision of biomedical information. One possible explanation for this seemingly contradictory result is that GPs react to patient concerns with the provision of medical information, hoping that this might alleviate their worries. We have checked this possibility with additional sequential analysis, but found that the provision of biomedical information actually occurs less after patients' expression of cues or concerns. Another possibility is that the positive relationship between physician biomedical information giving and patient concerns in the cross-sectional analysis is a spurious relationship, which means that both variables are influenced by a third variable. Indeed, when testing for consultation length as confounding variable, this relationship disappeared. This means that in longer medical encounters, physicians tend to give more biomedical information and patients tend to offer more cues or concerns, but this is not necessarily related. On the contrary, the sequential analyses clearly show that if GPs present biomedical information in a monologue, without giving room to the patient by facilitative behavior, eye contact and psychosocial questions, there is a fair chance that patients will not express their concerns. From a clinical viewpoint, this means that physicians should regularly interrupt their stream of information to provide room for the patient to give a reaction from their own life world. Only in this way can the voice of medicine and the voice of patients' life world be integrated within the medical dialogue.7

This study has some limitations. An important limitation is that only physician communication that immediately preceded the discussion of patient concerns was analyzed. Clearly, events preceding that contiguous conversation element could have influenced a patient's openness about concerns. However, an important reason for not choosing longer sequences is that several studies have shown that the first “lag” shows the strongest relationship with the target variable, whereas effects tend to extinguish rather soon.12,30 Another reason for choosing short sequences has a more theoretical background which stems from CA.37,38 In CA, turn taking, adjacency pairs, and repair are central concepts.38 According to CA, a medical encounter evolves step-by-step in a social interaction in which each participant reacts continuously on the contribution of the other participant. In this way, both participants coconstruct the medical interview, using the reaction of their partner to “repair” their original statement if necessary. This concept of “repair” is the reason why we have chosen for 2, instead of 1 preceding physician utterance. However, posthoc analyses showed that the results would have been the same if we had used only 1 preceding utterance.

Another possible limitation of the study is the lack of context information, which could be confounding the results. However, in our study design, we have taken care for several potential

confounders. We applied multilevel analysis to control for the clustering of communication sequences within medical encounters. We also addressed 2 common problems in sequence analysis (the stationarity problem and the homogeneity problem 23) in our study design. The stationarity problem, which means that communication can evolve over time, resulting in different types of communication sequences, has been addressed by repeating all analyses for the first cue or concern only. The fact that these repeated analyses show the same results as the overall analyses gives credibility to the reported results. The risk of a potential homogeneity problem has been addressed by restricting the analyses to a homogeneous patient group: hypertensive patients.

Another limitation follows directly from our choice for a homogenous patient sample: the study is restricted to GPs' medical encounters with hypertensive patients. This means replication studies within other medical settings and with other types of patients are needed before the results can be generalized. However, for this patient group, the results are highly relevant, because it has been demonstrated that hypertensive patients—just like many other chronically ill patients—have many concerns, for instance, about the consequences of their illness for daily life, while physicians tend to restrict themselves to biomedical issues only.

Despite the limitations, we believe that results of this study are relevant for researchers and clinicians. For researchers, the value of the study lies primarily in the methodological innovation and reflections. For clinicians, it is important to know that patients are less inclined to disclose their worries when the physician is presenting biomedical information. Because physicians have to give lots of biomedical information during most medical encounters, we recommend to avoid long monologues, but to build in pauses, in which silence, eye contact, facilitative communication, and psychosocial questions are used to provide room for patients to disclose their concerns.

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Key Words: communication; sequence analysis; cues; concerns; eye contact; patient participation

TABLES

TABLE 1. Cross-Sectional Analyses: The Relationship Between GP Communication and Patients' Expression of Cues and Concerns Estimated With Poisson Regression

GP Communication, N = 99	Cues, Exp(b)	Concerns, Exp(b)	Cues + Concern, Exp(b)
Task oriented	1.08***	1.13**	1.09***
Biomedical questions	1.27	1.61	1.34
Biomedical information	1.08*	1.15*	1.10*
Biomedical counseling	1.08	1.21	1.11
Psychosocial questions	2.75***	1.55	2.42**
Psychosocial information	1.30*	1.42	1.33*
Psychosocial counseling	2.19	1.00	1.88
Lifestyle questions	1.09	0.66	0.97
Lifestyle information	1.12	1.11	1.12
Lifestyle counseling	1.40	0.46	1.13
Affect oriented	1.12***	1.17***	1.13***
Social talk	0.90	0.72	0.86
Concern/optimism	1.48	1.07	1.39
Facilitation	1.18***	1.28***	1.21***
Process oriented	0.95	1.15	1.00
Instructions	0.90	1.12	0.95
Seeking dialogue	1.74	2.38	1.88
Disagreements	1.96	6.36	2.70
Other	0.96	0.87	0.93
% Patient participation	1.03*	1.06*	1.05*
% Eye contact	1.02***	1.02*	1.02***

Univariate Poisson regression models allowing for extra Poisson variance. N = 99. Coefficients are considered significant when $P < 0.05$.

Exp(b) indicates the factor of change in frequency of cues or concerns at a 10 utterance (RIAS) or 1% (patient participation and eye contact) change in the predictor variable.

*$P < 0.05$.
**$P < 0.01$.
***$P < 0.001$.

TABLE 2. Sequential Analyses: The Probability of Patients' Cues or Concerns being Preceded by Patient Talk Estimated With Logistic Regression

Dependent Variable	b	SE	OR
First cue	3.91	0.79	50.10***
First concern	1.45	0.84	4.26
First cue or concern	2.93	0.71	18.67***
All cues	1.86	0.37	6.45***
All concerns	1.41	0.61	4.09*
All cues and concerns	1.77	0.32	5.88***

Coefficients are considered significant when $P < 0.05$.

Independent variable is the proportion of patient utterances in the preceding 5 utterances. The OR indicates how much the probability of patients' expressing a cue or concern increases when all 5 utterances are expressed by the patient (versus all are expressed by the GP).

*$P < 0.05$.

***$P < 0.001$.

TABLE 3. Sequential Analyses: Probabilities of Patients' Expression of Cues and Concerns When Preceded by Specific GP Communication Estimated With Logistic Regression

Preceding GP Communication, N = 11,794 Utterances	OR		
	Patients' Cues	Patients' Concerns	Patients' Cues or Concerns
Task oriented	0.69***	0.67*	0.67***
Biomedical questions	1.00	1.03	0.98
Biomedical information	0.53***	0.26***	0.45***
Biomedical counselling	0.40*	0.36	0.39**
Psychosocial questions	2.68***	6.47***	3.50***
Psychosocial information	1.00	1.34	1.05
Psychosocial counselling	0.76	1.84	0.97
Lifestyle questions	1.36	1.29	1.07
Lifestyle information	0.20	0.74	0.31
Lifestyle counselling	0.77	—	0.63
Affect oriented	1.89***	2.05***	1.97***
Social talk	0.55**	0.34	0.49***
Concern/optimism	1.66	—	1.31
Facilitation	2.16***	2.64***	2.33***
Process oriented	0.47***	0.45	0.47***
Instructions	0.40***	0.30*	0.38***
Seeking dialogue	0.88	1.08	0.97
Disagreements	1.07	3.07	1.62
Other	0.57	0.31	0.53*

Coefficients are considered significant when $P < 0.05$.

OR depicts the change in probability of patients expressing a cue or concern following the listed GP communication versus all other sorts of GP communication. For each patient utterance, the 2 GP utterances directly preceding patient communication are taken as predictor variables.

*$P < 0.05$.

**$P < 0.01$.

***$P < 0.001$.

TABLE 3. Sequential Analyses: Probabilities of Patients' Expression of Cues and Concerns When Preceded by Specific GP Communication Estimated With Logistic Regression

Preceding GP Communication, N = 11,794 Utterances	OR		
	Patients' Cues	Patients' Concerns	Patients' Cues or Concerns
Task oriented	0.69***	0.67*	0.67***
Biomedical questions	1.00	1.03	0.98
Biomedical information	0.53***	0.26***	0.45***
Biomedical counselling	0.40*	0.36	0.39**
Psychosocial questions	2.68***	6.47***	3.50***
Psychosocial information	1.00	1.34	1.05
Psychosocial counselling	0.76	1.84	0.97
Lifestyle questions	1.36	1.29	1.07
Lifestyle information	0.20	0.74	0.31
Lifestyle counselling	0.77	—	0.63
Affect oriented	1.89***	2.05***	1.97***
Social talk	0.55**	0.34	0.49***
Concern/optimism	1.66	—	1.31
Facilitation	2.16***	2.64***	2.33***
Process oriented	0.47***	0.45	0.47***
Instructions	0.40***	0.30*	0.38***
Seeking dialogue	0.88	1.08	0.97
Disagreements	1.07	3.07	1.62
Other	0.57	0.31	0.53*

Coefficients are considered significant when $P < 0.05$.

OR depicts the change in probability of patients expressing a cue or concern following the listed GP communication versus all other sorts of GP communication. For each patient utterance, the 2 GP utterances directly preceding patient communication are taken as predictor variables.

*$P < 0.05$.

**$P < 0.01$.

***$P < 0.001$.