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Original Article

Clinical characteristics and types of *EGFR* gene mutations in patients with synchronous primary lung cancer

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Abstract: Background: Synchronous primary lung cancer (SPLC) is a rare condition. Limited information regarding SPLC has been reported in the literature. No guidelines regarding optimal diagnosis or treatment of SPLC currently exist. The purpose of this study was to provide insight into the characteristics of patients, lesions, molecular biomarkers, and associated mutations in epidermal growth factor receptor (EGFR) in SPLC. Methods: This study reviewed 21 cases of SPLC, from 1999 to 2017, and information regarding the clinical characteristics, molecular biomarkers, and gene mutations was reported. Results: Of the 21 cases, the male to female ratio was 14:7, heavy smokers (n = 11, 52.4%) were predominant, and most of the patients were 60 years old or older. A total of 49 tumors were found in these 21 patients. The majority of patients had bilateral cancer (66.7%) and most of the tumors presented with different pathological patterns (71.4%). SPLC was more prevalent in the upper lobe (57.1%) as opposed to the lower lobe (26.5%). Adenocarcinoma was the most common pathological pattern (40.8%), followed by squamous cell carcinoma (22.4%). A total of 23 lesions (46.9%) received surgical intervention. Seven common positive antigens were found, including CD56, CK7, CK14, Ki-67, TTF-1, synaptophysin, and chromogranin, among the 21 patients with SPLC. Small cell lung cancer (SCLC) and neuroendocrine tumors were more likely be positive for these biomarkers than other pathological patterns. Deletion of exon 21 of *EGFR* genes was more prevalent in SPLC presenting in the upper lobe than in the lower lobe. Elderly men with adenocarcinoma were more likely to have an EGFR mutation, among patients with SPLC. Conclusion: This current analysis suggests that elderly men that smoke have higher risk of SPLC. Moreover, SCLC and neuroendocrine are also more likely to be positive for related molecular biomarkers than other pathological patterns. Additionally, adenocarcinoma is more likely to present with an EGFR mutation among these patients.

Keywords: Synchronous, lung cancer, primary, clinical characteristics

Introduction

In both men and women, lung cancer is the leading cause of cancer-related deaths. In 1975, Martini and Melamed [1] introduced clinicopathological criteria for diagnosis of multiple primary lung cancer (MPLC) and, unfortunately, despite the first diagnostic criteria being proposed in the 1970s [1], no guidelines regarding optimal diagnosis and treatment of SPLC currently exist. Incidence of synchronous primary lung cancer (SPLC) ranges from 0.2 to 8% [2]. It is very difficult to distinguish SPLC from multicentric lung cancers or from metastatic lung cancer with intrapulmonary or pulmonary metastases that have originated from primary cancers in different organs. However, it is clinically important for staging, establishing a management plan, and prognosis. Histology alone may not distinguish between metastatic deposits and independent tumors [3]. In such situations, molecular and/or gene analysis may contribute to obtaining an accurate diagnosis [4].

Previous case reports have stated that mutation of the epidermal growth factor receptor (EGFR) genes and other positive molecular biomarkers may help in differentiating SPLC from

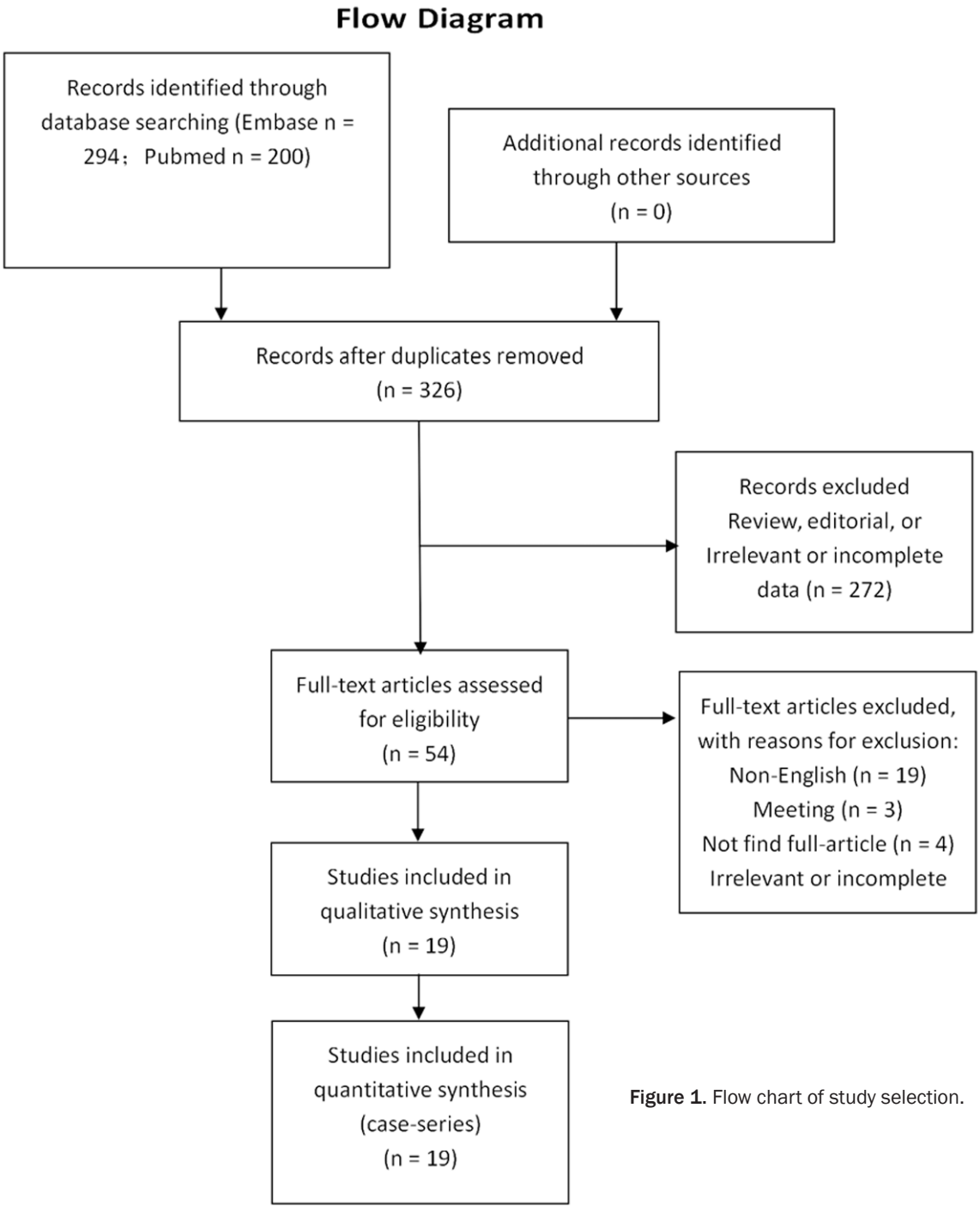

Figure 1. Flow chart of study selection.

metastatic lesions. In the present study, a case series and literature review of SPLC is presented. The purpose of this study was to provide insight into the characteristics of patients, lesions, molecular biomarkers, and EGFR mutations among patients with SPLC, summarizing a total of 21 cases.

Methods

Search strategy

Search of the available literature was carried out using PubMed database (from January 1966 to February 2018) and Embase database

Table 1. Clinical characteristics of patients

Characteristics	Data
Total	21
Gender	
Male	14 (66.7%)
Female	7 (33.3%)
Heavy smoker	11 (52.4%)
Age (years old)	
50-60	2 (9.5%)
61-70	10 (47.62%)
71-80	9 (42.9%)
Symptom	
Positive symptom	12 (57.1%)
No (routine checkup find)	9 (42.9%)
Location	
Ipsilateral	7 (33.3%)
Bilateral	14 (66.7%)
Pathological pattern	
Same	5 (23.8%)
Aadenocarcinoma	5 (100%)
Different	15 (71.4%)
Ad-sclc-neur	1 (6.7%)
Ad-sclc-sq	1 (6.7%)
Ad-sq-neur	1 (6.7%)
Ad-sq	3 (20%)
Sq-sclc	3 (20%)
Sq-bronchiolo-alveolar cell carcinoma	1 (6.7%)
Sq-neur	1 (6.7%)
Sq-sarcoma	2 (13.3%)
Ad-pleomorphic carcinoma	1 (6.7%)
Sclc-nsclc	1 (6.7%)
Other	1 (4.76%)

Note: Ad, adenocarcinoma; SCLC, small cell lung cancer; neur, neuroendocrine; Sq, squamous; nsclc, non-small cell lung cancer.

(from 1947 to 2018). Key search terms used were: synchronous, primary, and lung. All publication types, races, and medical subcategories were included in the search.

Study selection

Studies were reviewed by two independent reviewers, screening titles and abstracts to evaluate the contents of potentially eligible studies. Disagreements were resolved by a consensus vote. All included patients were diagnosed as having SPLC by pathological examination.

Data extraction

For included studies, two investigators independently carried out data extraction, moving the data it into a Microsoft Excel (2010) file without notifying each other until both tasks were completed. Information was collected regarding age at presentation, gender, symptoms and signs, smoking, radiological finding (e.g., MRI, X-ray, and CT), lesion locations, pathological pattern, treatment strategy, lesion size, relative molecular biomarkers, and EGFR mutation type.

Statistical analysis

Statistical analysis was carried out using SPSS statistical software, version 19.0 (SPSS, Inc., Chicago, IL, USA). Categorical variables are reported as counts and percentages while continuous variables are presented as mean ± SD. Differences in proportions were statistically evaluated using Chi-square test. All statistical tests were two-tailed and a *P*-value of less than 0.05 was considered statistically significant.

Results

Case selection

As presented in **Figure 1**, following comprehensive literature retrieval from the databases regarding treatment for SPLC, a total of 494 records were initially identified as eligible. Following a scan of the abstracts, 272 irrelevant studies were excluded, being reviews, editorials, or containing irrelevant or incomplete data. A total of 35 were also excluded following a complete reading, including studies not written in English, meeting reviews, and irrelevant reports. Lastly, 19 case reports [5-23], published between 1999 and 2017, were included in this case series.

Patient characteristics

A total of 21 patients with SPLC were enrolled this study from 19 case reports (data were shown in Table S1). Descriptive characteristics including sex, age, smoking status, tumor location, and pathological pattern were included in the analysis and are summarized in **Table 1**. There were more male (*n* = 14, 66.7%) than female patients (*n* = 7, 33.3%) and heavy smokers (*n* = 11, 52.4%) were predominant. Two

Table 2. Characteristics of the lesions

Clinical characteristics	Data
Total	49 (100%)
Location	
LLL	5 (10.2%)
LUL	17 (34.7%)
RLL	8 (16.3%)
RUL	11 (22.4%)
RML	4 (8.2%)
Bronchiolar origin	1 (2.0%)
Endobronchial	2 (4.1%)
Left main bronchus	1 (2.0%)
Pathological pattern	
Adenocarcinoma	20 (40.8%)
SCLC	7 (14.3%)
Neuroendocrine	3 (6.1%)
Squamous cell carcinoma	11 (22.4%)
Bronchiolo-alveolar cell	1 (2.0%)
Carcinosarcoma	2 (4.1%)
NSCLC	1 (2.0%)
Pleomorphic carcinoma	3 (6.1%)
Others	1 (2.0%)
Treatment	
Surgery	23 (46.9%)
Segmentectomy	2 (8.7%)
Lobectomy	11 (47.8%)
Wedge resection	3 (13.0%)
Others	7 (30.4%)
Surgery + chemotherapy	6 (12.2%)
Lobectomy + chemotherapy	2 (33.3%)
Wedge resection + chemotherapy	1 (16.7%)
Other surgery + chemotherapy	3 (50%)
Chemotherapy	11 (22.4%)
Other treatments	9 (18.3%)
Size (cm)	
(0-1]	2 (4.1%)
(1-2.5]	13 (26.5%)
(2.5-5]	10 (20.4%)
> 5	2 (4.1%)
Not applicaple	23 (46.9%)

Note: LLL, left lower lobe; LUL, left upper lobe; RML, right middle lobe; RUL, right upper lobe; RLL, right lower lobe; SCLC, small cell lung cancer; NSCLC, non-small cell lung cancer.

(9.5%) patients were 50-60 years of age, 10 (47.62%) were 61-70 years of age, and 9 (42.9%) were 71-80 years of age. Patients ≥ 60 years old were the majority. No patients were less than 50 years old. Only 12 (57.1%) patients presented with obvious symptoms when diagnosed with SPLC. The remaining 9 (42.9%) patients received diagnosed of this disease following routine checkup with no prior abnormal signs. Most patients had bilateral cancer (66.7%), with 7 (33.3%) patients having ipsilateral cancer. Most patients presented with different pathological patterns (71.4%), while the remaining 5 (23.8%) patients presented with the same pathological pattern among multiple tumors, all of which were adenocarcinoma.

Basic tumor characteristics

Basic tumor characteristics are shown in **Table 2**. A total of 49 tumors were found in these 21 patients. In total, 5 (10.2%) tumors were located in the left lower lobe, 17 (34.7%) in left upper lobe (LUL), 8 (16.3%) in right lower lobe (RLL), 11 (22.4%) in right upper lobe (RUL), and 4 (8.2%) in right medial lobe (RML). One lesion was bronchiolar in origin, 2 (4.1%) were endobronchial, and 1 was of the left main bronchus. Adenocarcinoma was the most common pathological pattern (20; 40.8%), followed by squamous cell carcinoma (11; 22.4%) and small cell lung cancer (7; 14.3%). Other patterns, including neuroendocrine, bronchioloalveolar cell, carcinosarcoma, and non-small cell lung cancer (NSCLC), were relatively less common (6.1%, 2.0%, 4.1%, 2.0%, and 6.1%, respectively). In total, 23 lesions received surgical intervention. Lobectomy (11; 47.8%) was the most frequently used surgical procedure, followed by wedge resection (3; 13.0%) and segmentectomy (2; 8.7%). Surgery and chemotherapy or chemotherapy alone was also used to treat some patients, including 6 (12.2%) and 11 (22.4%) tumors, respectively. However, detailed treatment strategies were not mentioned for the remaining tumors. A total of 2 tumors were less than 1 cm in size, 13 (26.5%) were 1-2.5 cm, 10 (20.4%) were 2.5-5 cm, and 2 were more than 5 cm. However, size information of 23 (46.9%) tumors was not indicated.

Tumor immunochemical characteristics

Common molecular biomarkers are shown in **Table 3**. CD56 was positive in 3 SCLC and 2 neuroendocrine tumors. CK7 in was positive 1 SCLC and 2 adenocarcinoma tumors. CK14 was positive in 1 neuroendocrine and squamous cell carcinoma tumor. Ki-67 was positive in 2 SCLC tumors. TTF-1 (thyroid transcription

Table 3. Immunochemical characteristics in tumors

	CD 56 (n= 5)	CK 7 (n=3)	CK 14 (n=2)	Ki-67 (n=2)	TTF-1 (n=4)	Synaptophysin (n=4)	Chromogranin (n=5)
Pathology							
SCLC	3	1	0	2	2	3	4
Neuroendocrine	2	0	1	0	0	1	1
Adenocarcinoma	0	2	0	0	1	0	0
Squamous cell carcinoma	0	0	1	0	1	0	0
Location							
Endobronchial	1	0	1	0	0	0	0
LLL	2	1	1	1	1	1	1
LUL	1	1	0	0	2	1	2
RML	0	0	0	1	1	1	0
RUL	0	1	0	0	0	0	1
RLL	0	0	0	0	0	1	1

Note: SCLC, small cell lung cancer; LLL, left lower lobe; LUL, left upper lobe; RML, right middle lobe; RUL, right upper lobe; RLL, right lower lobe; TTF-1, thyroid transcription factor 1. Note: n present the number of positive.

Table 4. Association of *EGFR* mutation with tumor location and pathological pattern among three subtypes

	Exon 19 deletion (n=4)	Exon 21 deletion (n=2)	Wild-type (n=1)	*P* value
Tumor location				0.675
Upper lobe	1	2	0	
Lower lobe	2	0	1	
Middle lobe	1	0	0	
Pathology				1
Adenocarcinoma	2	1	1	
Bronchiolo-alveolar cell carcinoma	1	0	0	
Others	1	1	0	
Sex				1
Male	3	1	1	
Female	1	1	0	
Age (years)				0.619
60-70	2	0	0	
70-80	2	2	1	

factor 1) was positive in 2 SCLC, 1 adenocarcinoma, and 1 squamous cell carcinoma tumor. Synaptophysin was positive in 3 SCLC and 1 neuroendocrine tumors and chromogranin was positive in 4 SCLC and 1 neuroendocrine tumor.

EGFR gene mutations

Further characterization of tumors, according to *EGFR* mutation status, is presented in **Table 4**. Among EGFR activating mutations, deletion of exon 21 was more prevalent in upper lobe than lower lobe tumors. Compared with other pathological patterns, adenocarcinoma (57.1%) was more likely to have an *EGFR* mutation. A total of 5 (71.4%) of the 7 tumors with gene mutations were obtained from male patients older than 70 years. Overall distribution of tumor locations and pathological patterns among the three gene mutation subtypes were not significantly different.

Discussion

Synchronous primary lung cancer is a rare disease. Nevertheless, it is not easy to distinguish SPLC from metastatic lung cancer with intrapulmonary or pulmonary metastases originating

from primary cancers in different organs. Therefore, clinical features of patients and tumors, positive biomarkers, and existing gene mutations may provide valuable clues illuminating this distinction. This present study performed a case series with 21 patients based on 19 case reports [5-23], summarizing lesion characteristics, positive molecular biomarkers, and types of *EGFR* gene mutations.

Definite etiology and clinical manifestations of SPLC have not been reported due to the limited number of cases. This present study found that male patients outnumbered female patients, heavy smokers were predominant, and patients more than 60 years old were significantly in the majority. Thus, this study suggests that men older than 60 years that smoke are more likely to present with SPLC. Only 57.1% of these patients presented with obvious symptoms at diagnoses of SPLC and 42.9% of patients received their diagnoses during routine check-up with no prior abnormal signs. Therefore, routine checkups might play an important role in detecting SPLC at an early stage.

Lung cancers usually have a single histological type, however, there have been reports of patients with MPLC with different histologies. Most patients in this study presented with different pathological patterns (71.4%). However, Ferguson [24] summarized that about 48.7% of individual patients have different histologic subtypes, lower than the present findings. They also found that squamous cell carcinoma was the most common cancer, comprising over 70% of synchronous cancers with identical histologic subtypes and nearly 85% of patients with tumors of different histologic subtypes [24]. This report is in contrast to the present results indicating that adenocarcinoma comprised all of the synchronous cancers with the same pathological pattern. Previous studies have found that, based on tumor location in the lungs, bilateral cancer comprises 60-70% while unilateral cancer is comparatively rare [25, 26]. In this present study, most patients had bilateral cancer (66.7%) and only 7 (33.3%) patients had ipsilateral cancer. However, only patients with double primary lung cancers, rather than other multiple cancers, were included in the prior study [24]. This fact, along with the limited sample size of the present study, might be reasons for these discrepancies.

This present study summarized tumor characteristics from these patients, concluding that SPLC is more prevalent in the upper lobe than lower lobe. To the best of our knowledge, there have been no prior studies reporting this observation. Some studies have suggested that surgery should not be performed if tumors have advanced beyond stage II [1, 27]. However, Trousse suggested that surgery, after an appropriate selection process, is of benefit for patients with SPLC. In patients with multiple synchronous lesions, limited surgical procedures to remove lesions have been preferred, especially since pneumonectomy has been associated with increased risk of death [28]. Results of the current study indicate that about 60% of lesions received surgical intervention, with lobectomy (47.8%) the most frequently used surgical procedure. These findings confirm the above supposition but present researchers were not able to obtain information regarding the benefit of these procedures for patients due to the absence of follow ups.

Several authors have proposed the use of molecular analysis, along with clinicopathological characteristics, to distinguish multiple primary tumors from intrapulmonary metastases [29-31]. It has been previously reported that profiling expression of several antigens, including CK19, p53, CEA, Hup-1, PE-10, and Ki-67, is useful in distinguishing multiple primary cancers from pulmonary metastases in cases with adenocarcinoma [32]. This present study found seven common positive antigens, including CD56, CK7, CK14, Ki-67, TTF-1, synaptophysin, and chromogranin, among the 21 patients with SPLC. Moreover, SCLC and neuroendocrine were more likely to present as positive for these biomarkers than other pathological patterns. However, appropriate antigens should be further defined before use in clinical application.

Mutation of EGFR is a common early event in lung cancer pathogenesis. Therefore, it is a useful marker for differentiating the clonal origin of lung tumors, especially when multiple tumors have similar histopathological features [33]. This current study found three common types of EGFR mutations, summarizing the tumor characteristics according to mutation status for these 21 cases. It was concluded that deletion of exon 21 was more prevalent in upper lobe lesions than in lower lobe lesions

and that elderly men with adenocarcinoma were more likely to have an EGFR mutation. Thus, it was surmised that it could be beneficial to assess the presence of EGFR gene mutations in elderly male patients with SPLC. However, statistical differences among the three genotype groups were not obtained. These results were just tendency, a limitation of the present research. Too few cases might the explanation for this result. Many more studies are needed to elucidate this question.

Conclusion

This current analysis suggests that men over 60 years old, that also smoke, have higher risk of developing SPLC. Importantly, SCLC and neuroendocrine tumors are more likely to be positive for related molecular biomarkers than other pathological patterns. Additionally, among patients with SPLC, adenocarcinomas are more likely to present with an EGFR mutation.

Disclosure of conflict of interest

None.

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Table S1. Main characteristics of each case report

No	First author	Year	Country	Age	Sex	Symptom	Heavy smoking	Duration
1	Kashif	2017	USA	63	M	Sharp chest pain, shortness of breath and chronic dry cough	Yes	5 ms
2	Zardo	2014	Germany	72	F	Routine checkup	Yes	2 ys
3	Froio	2007	Italy	59	M	Routine checkup	NA	NA
4	Yoon	2014	Korea	72	M	Routine checkup	NA	NA
5	Kontic	2011	Serbia	60	F	Routine checkup	yes	NA
6	Ma	2010	China	75	M	Routine checkup	NA	NA
7	Ryoo	2006	KOREA	66	M	chronic cough	Yes	6 ms
8	Dohmoto	1999	Japan	69	F	Routine checkup	NA	NA
9	Umemura	2011	Japan	74	M	Cough	Yes	1 m
10	Lin	2010	China	82	M	Cough with blood-streaked phlegm	Yes	2 ws
11	Seo	1991	Korea	72	M	Cough, sputum, and mild dyspnoea	Yes	NA
12	liu	2014	China	78	F	Persistent cough	No	NA
13	liu	2014	China	62	M	Persistent cough and hemoptysis	Yes	1 week
14	liu	2014	China	73	M	Persistent cough with sputum	Yes	2 ms
15	Yu	2015	China	62	F	Routine checkup	No	NA
16	Liu	2014	China	67	F	Chest pain	NA	2ms
17	Takuwa	2010	Japan	78	F	Routine checkup	NA	NA
18	Bacalja	2016	Croatia	58	M	Intermittent morning cough with blood impurities	Yes	Few months
19	Taira	2014	Japan	64	M	NA	NO	NA
20	Wang	2016	China	66	M	Dry cough accompanied with blood in phlegm	Yes	1 month
21	Graziano	2011	Italy	77	M	Routine checkup	NO	NA